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A STUDY OF *BACTERIUM LINENS*[1]

J. OSCAR ALBERT

From the Department of Dairy Industry, Iowa State College

During the ripening of certain cheeses, a reddish-brown slimy growth commonly develops on the surfaces. The material contains various microorganisms, among which *Bacterium linens* usually is present in relatively large numbers. This organism apparently plays a role in the ripening of certain cheeses, in which it aids in the protein breakdown and flavor development; it also aids in the production of the typical color at the cheese surfaces.

The work herein reported involved: (1) Development of an isolation procedure for *Bact. linens*, (2) studies on its distribution in dairy products and other materials, (3) studies on the general properties of the organism, and (4) preparation of a description of the organism.

The medium developed during the studies and used in making isolations has the following composition:

Ripened cheese	10.0 per cent
Potassium citrate	1.0 per cent
Peptone	1.0 per cent
Sodium chloride	5.0 per cent
Sodium oxalate	0.2 per cent
Agar	1.5 per cent
Water to make	100.0 per cent

pH 7.4

The materials to be examined for *Bact. linens* are smeared on surfaces of plates poured with the agar, either directly or after dispersion in sterile water. The plates are placed under a bell jar or otherwise enclosed, and oxygen is allowed to run into the container slowly for 15 minutes or more, depending on the number of plates to be incubated. The inlet and the outlet tubes are then closed; although 21°C. is a good temperature for incubation, room temperature has been extensively employed and ordinarily gives good results. One week usually is required for good growth and for complete development of color. By using the special cheese agar and following the incubation procedure, *Bact. linens* develops readily. The color production is much more intense and characteristic than when tryptone glucose extract agar or similar media are used with the plates incubated in air and, together with the general colony appearance, permits the detection of the organism with a high degree of accuracy.

The distribution of *Bact. linens* was studied by examining dairy products and various materials from dairy farms and other sources with the general isolation procedure suggested for the organism. A total of

[1] Doctoral thesis number 725, submitted July 9, 1943.

51 samples of foreign type cheeses were investigated and most of them yielded *Bact. linens*. Of 35 samples of cheddar cheese made with raw milk, a rather large percentage contained the organism in relatively small numbers. *Bact. linens* also was found in some of a small number of cheese made from pasteurized milk. The organism was recovered from more than one-half of the samples of milk and cream examined. It was found in most of 59 samples of various kinds of feeds, including corn, oats, barley, and wheat. Certain samples of silage yielded *Bact. linens*. Green plants, hay, and straw yielded it in about one-half of the instances. The organism was recovered rather regularly from water used for watering cows or standing in the barn yards. Mouths of cows yielded *Bact. linens* in several instances when the cows were in the barn, while the organism was not found when the cows were on pasture. *Bact. linens* was obtained from more than one-half of the samples of manure. It was found in the air of various dairy plants, cheese factories, stables, etc. The presence of *Bact. linens* in cheddar cheese made from pasteurized milk may have been due to the organism falling from the air into the milk or the curd. *Bact. linens* was not found in soil.

In litmus milk, *Bact. linens* produced an alkaline reaction and then conspicuous proteolysis. On extended incubation it greatly increased the soluble nitrogen in milk, but different strains varied considerably in the extent of the proteolysis. Amino nitrogen was significantly increased, as were also the fraction soluble in trichloracetic acid and the fractions soluble and insoluble in ethyl alcohol or phosphotungstic acid.

The organism was not lipolytic, but in unsalted butter at 21°C. a putrid condition was produced.

Color production on tryptone glucose extract agar by *Bact. linens* was increased by adding 10 per cent peptone or 5 per cent peptone and 5 per cent casein.

In a medium consisting of 0.3 per cent desiccated yeast extract in water, *Bact. linens* produced volatile acids from various alcohols. Ethyl alcohol yielded practically only acetic acid; propyl alcohol yielded largely propionic acid, and there was evidence of some other acid; butyl alcohol yielded essentially only butyric acid; and amyl alcohol yielded largely valeric acid with a trace of some other acid. In the medium under the conditions used, hexyl and heptyl alcohols yielded very little volatile acid.

In 2 per cent peptone solutions, *Bact. linens* grew at a pH of approximately 6.0 but not at a pH of approximately 5.0. It also grew at a pH of approximately 9.8. In litmus milk in the presence of *Streptococcus lactis*, *Bact. linens* decreased in numbers rather rapidly.

In litmus milk, *Bact. linens* survived for at least 4 months at room temperature. When dried on filter paper it survived for at least 3 months.

The organism grew in the presence of large amounts of sodium chloride; all strains grew in skim milk saturated with it, and some grew in peptone solution saturated with it.

Bact. linens was rather easily destroyed by heat. Only two of the

strains survived 62.8°C. for 5 minutes, which was the shortest exposure used.

All the strains of *Bact. linens* examined were strongly catalase positive.

A description of the organism was prepared.

SOME APPLICATIONS OF ELECTROMETRIC METHODS TO THE STUDY OF THE COMPONENTS OF STARCH[1]

FRANCIS LESLIE BATES

From the Department of Chemistry, Iowa State College

In recent years investigations in the field of starch chemistry have provided considerable support for the idea that natural starches are composed of two distinct components. Three methods of effecting their separation have been developed (1, 2, 3), and although the mechanism responsible for the separation is different in each procedure, the fractionations that result are quite similar. In each case the starch is separated into two fractions. One gives a deep blue color with iodine, is converted almost completely to maltose by β-amylase, possesses a higher reducing value than does whole starch, and gives crystalline X-ray diffraction patterns upon retrogradation or upon precipitation with alcohol. The other fraction stains purple to red with iodine, is only partially converted by β-amylase, has a low reducing power, retrogrades with difficulty, and produces very poor or amorphous X-ray patterns. Meyer should be given the major share of the credit for perceiving the nature of the fundamental difference between the two components. He has published reviews (4, 5) of the work that have led him to the conclusion that the fraction which stains blue with iodine consists of unbranched starch molecules. The second fraction he identifies with branched chain material, thus limiting to only one component a structure that had been proposed for whole starch by earlier investigators. Meyer calls the unbranched component "amylose" and the branched "amylopectin."

The difference in behavior toward iodine exhibited by the two starch components is easily established in a qualitative manner. The present investigation was initiated by experiments designed to place this behavior on an exact quantitative basis. It was found that the iodine electrode, consisting simply of a bright platinum wire immersed in a solution containing free iodine and iodide ions, provided a very sensitive instrument for measuring changes in iodine activities. Titration of an amylopectin solution with a dilute iodine solution resulted in a steadily increasing iodine activity. In the case of amylose solutions, however, the iodine activity remained almost constant until an amount of iodine had been added equal to about one-fifth the weight of amylose present. The subsequent increase in iodine activity produced an inflection point similar to that obtained in the usual potentiometric titration. The amount of iodine bound by a pure amylose sample under certain well-defined conditions was carefully determined. The great difference in the iodine-binding abilities of amylose and amylopectin was made the basis of an analytical method by which

[1] Doctoral thesis number 729, submitted August 18, 1943.

the amylose content of mixtures of the two components could be determined. Successful in the analysis of known mixtures, it was applied to whole starches and crude starch fractions. The behavior of the two components can best be explained on the basis of a helical configuration of the starch in the complex. This has been confirmed by Rundle and coworkers (6, 7, 8).

Potentiometric iodine titration of starch fractions was used to prove that starch was composed of only two distinctly different components. While the fractionation procedures did not produce complete separation of the two, titration of the fractions showed that one of the methods produced a very good separation and also showed that the crude fractions could be purified effectively. No material was found that had an iodine-binding ability intermediate between those possessed by amylose and amylopectin. The conclusion was drawn that no component existed with structure intermediate between the unbranched chains of the amylose fraction and the highly branched molecules of amylopectin.

The results obtained in the iodine titration of amyloses indicated that longer amylose chains bound iodine at a lower iodine activity than did short ones. It was also found that very short amylodextrins required such high iodine activities that the shortest ones were indistinguishable from amylopectins. This behavior of the amyloses permitted them to be arranged according to their relative molecular weights. It also showed that the amylose fractions obtained from natural starches were quite homogeneous as to chain length. Starch synthesized from glucose-l-phosphate by action of phosphorylase appeared to be a heterogeneous amylose.

The slight affinity of the amylopectins for iodine was also studied, and

TABLE 1

AMYLOSE CONTENTS OF STARCHES

Starch	Amylose, %	Starch	Amylose, %
Waxy corn, defatted	0.5–1	Corn, defatted	26
Tapioca	18	Sago	27
Potato	22	Lily bulb	27, 34
Wheat	24	Pea	29

it was found to vary inversely with the degree of branching. Glycogen, which is more highly branched than amylopectin, had almost no affinity for iodine. Titration of limit dextrins indicated a higher degree of branching than was found in the corresponding amylopectins.

The amylose contents of whole starches determined by the iodine titration procedure showed considerable variation. Table 1 lists a number of starches with their amylose contents.

A number of factors were found to affect the starch-iodine complex formation. They thereby influenced the iodine titration results. The amount of iodine bound by amylose varies inversely with the iodide concentration in the solution presumably because iodide and tri-iodide ions

also enter into the complex with the iodine. Fatty acids and their alkali metal soaps inhibit the formation of the complex and decrease the amount of iodine bound. Since they are present in many whole starches, they must be removed before the iodine titration procedure can be applied. The iodine activity necessary for complex formation with a given amylose varies inversely with the concentration of the amylose itself.

One of the fractionation procedures mentioned above (3) depends apparently on the formation of a crystalline butanol-amylose complex similar in structure to the iodine-amylose complex. It was found that phenol, pyridine, and aniline formed analogous complexes with amylose.

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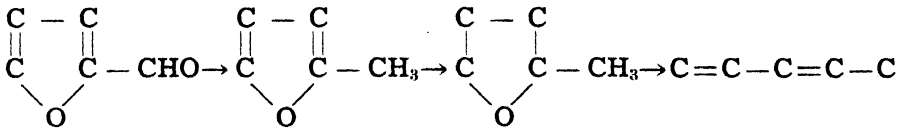
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THE PRODUCTION OF RUBBER FROM FURFURAL[1]

LLEWELLYN WILSON BURNETTE

From the Department of Chemistry, Iowa State College

The preparation of piperylene, or 1,3-pentadiene, from furfural has been undertaken with the view of developing a commercially practical process. Although other paths are possible, the following three steps were considered the most direct and feasible. During the course of this



study, a patent[2] was granted which covered essentially the same process, but the claims, particularly of the last two steps, were not substantiated in this laboratory.

Vapor phase catalytic reactions were utilized in the process. For the first two steps a vapor phase hydrogenation apparatus with a recirculation device was employed. This effected the saving of considerable quantities of hydrogen and uncondensed materials. The apparatus could also be employed for the dehydration in the last step.

More than twenty catalysts were studied for the conversion of furfural to methyl furan (sylvan). Only two types were found to be satisfactory. The copper deposited from the decomposition of copper acetate on a carrier such as activated charcoal was found to give yields of the order of 80–85 per cent consistently with one passage of the furfural over the catalyst. The best catalyst tried, however, was copper chromite dispersed on activated charcoal. This has been observed to give a yield of 95 per cent of sylvan in one passage. The optimum temperature observed for these catalysts was around 200°. It is interesting to note that copper chromite in the liquid phase produces furfuryl alcohol (or tetrahydrofurfuryl alcohol according to the conditions used) in quantitative amounts.[3]

Such an efficient conversion of furfural to sylvan applied commercially would make this compound available in large quantities at a low price. With such a stimulus, it should find many important industrial applications. It boils at 64°, has a refractive index of 1.433^{20} and a density of $0.916_{20}{}^{20}$.

The vapor phase hydrogenation of sylvan to tetrahydrosylvan was studied over several types of nickel catalysts. One of the more effective of these was partially activated Raney nickel. After determining that

[1] Doctoral thesis number 728, submitted July, 1943.

[2] H. Guinot (to Les Usines de Melle, Melle, Deux-Sevres, France), U. S. Patent 2,273,484, Feb. 17, 1942.

[3] Calingaert and Edgar, *Ind and Eng. Chem.*, 26: 878–881 (1934).

Raney nickel activated in the usual way led to ring opening and other undesired reactions, Raney nickel activated with only 6–8 per cent of the usual amount of NaOH was used with much better results. The best yields obtained, however, were of the order of 50 per cent. Besides the desired tetrahydrosylvan, pentanone-2 and pentanol-2 were identified in the product in appreciable quantities. Tetrahydrosylvan boils at 78°–79°, has a refractive index of 1.4059^{21} and a density of 0.8534_{15}^{21}.

The dehydration of tetrahydrofurans has been the subject of at least two patents,[2-4] in which yields of dienes as high as 85 per cent have been claimed. Although the reaction was not exhaustively studied in this laboratory, trials with five different dehydration catalysts have given yields up to only 30 per cent of piperylene. From the amount of water formed, more than twice this much dehydration was indicated, but it is presumed that the yield of the 1,3-fraction was decreased both by the formation of the isomeric 1,4-pentadiene and by decomposition of the desired product.

By carrying out the dehydration at a pressure of 60–85 mm., less decomposition and higher yields were obtained. In the case of the kaolin catalyst studied at a temperature of 400°, the yield of piperylene at atmospheric pressure was 17 per cent. At a lower pressure of about 70 mm. a yield of 30 per cent was obtained.

Piperylene (b.pt. 42°, $n\text{-}1.440^{16}$, d-0.696) was first observed to polymerize to a rubber by Thiele in 1901.[5] Since then, a limited amount of work has been reported in the literature regarding this property. Nothing current has appeared, however, which would serve to compare its properties with the present synthetic rubbers. This research is a necessary precursor to the application of the process on a commercial scale. In this laboratory an emulsion polymerization process similar to that used for butadiene has been used to prepare a satisfactory rubber from piperylene.

The potentially huge quantities of furfural annually available (estimated at 50,000,000 tons) have been emphasized in many instances. Assuming the utilization of but 21 per cent of the annual corn cob crop and mediocre yields in the reactions under discussion, it has been estimated that 100,000 tons of piperylene could be produced at a maximum cost of 38c a pound and at a probable cost much lower than this. This price compares favorably with the current prices of other synthetic rubbers.

In addition to the attractive price, the waste materials utilized in the furfural process are much less critical than the grain and petroleum resources now used. This, even more than cost considerations, should be the deciding factor in its adoption.

[4] Reppe, Steinhofer, and Hecht (to General Aniline and Film Corp.), U. S. Patent 2,241,792, May 13, 1941.
[5] Thiele, *Annalen, 319*, 226–30 (1901).

EFFECTS OF CARBON DIOXIDE AND OXYGEN ON ABSORPTION BY ROOTS [1]

HSIEN TSIU CHANG

From the Department of Botany, Iowa State College

The purpose of this study was to evaluate the relative importance of several factors which have been suggested as the causes of reduction of absorption by plant roots in insufficiently aerated media. Wheat, maize, and rice plants were grown in culture solutions under uniform conditions till about 5 to 7 weeks old, then divided into groups receiving different treatments. The treatments included bubbling (1) air, (2) carbon dioxide, or (3) nitrogen through the culture solutions for 10 minutes of each hour; (4) covering the solution with a thin layer of paraffin oil, and (5) controls with no treatment. The period of treatment lasted 36 hours covering approximately 24 hours of daylight and 12 hours of darkness. The amounts of water and nutrient elements absorbed by the plants under different treatments were determined and compared. In the experiment with rice plants, one series of the solutions was adjusted to an acidity of pH 4 by adding 1/10 N sulfuric acid to determine whether the effects of carbon dioxide were due to the increased acidity of the solution. The following results were obtained:

EFFECT OF CARBON DIOXIDE ON WATER ABSORPTION

Bubbling carbon dioxide through the culture solutions reduced water absorption by the three experimental plants by 14 to 50 per cent. In one experiment with wheat, bubbling nitrogen gas through the solution failed to bring about a reduction in water absorption. This indicates that the effect of carbon dioxide on the absorption is not due to its creation of a low oxygen concentration in the solution. The movement of water across membranes is considered to be physical diffusion with little or none

TABLE 1

WATER ABSORPTION UNDER DIFFERENT TREATMENTS. CK. IN ML. (EQUALS 100 PER CENT); OTHER TREATMENTS IN PERCENTAGE OF CK.

(Each Figure Represents an Average of 6 Replicates)

Crop	Experiment	Ck. (ml.)	Aerated %	Nitrogen %	Oiled %	Plus CO_2 %
Wheat	I	62.4	112.2			65.1
	II	53.5	106.0	112.9		73.8
Maize	I	33.1	117.5		98.8	85.8
	II	89.2	109.4		99.2	79.8
Rice	Acid+	133.9	99.4			54.8
	Acid−	139.4	111.5			50.2
Average %		100.0	109.3			68.3

[1] Doctoral thesis number 738, submitted December 16, 1943.

of the metabolic effects ascribed to mineral absorption. As a matter of fact water can be absorbed through dead root systems at temporarily increased rates, indicating a resistance factor. Water absorption was increased 9 per cent by aeration, as an average of all experiments. This increase could have been due to the stimulation effect of oxygen or to the removal of carbon dioxide. The failure of sulfuric acid, added to bring the control jars of the cultures to the same pH (4.0) as the CO_2-treated solutions, to significantly reduce water absorption, indicates that the effect of carbon dioxide is not solely through increasing the H-ion concentration of the solution.

EFFECT OF CARBON DIOXIDE ON THE ABSORPTION OF CATIONS AND ANIONS

Tables 2 to 6 inclusive give the effect of different treatments on the absorption of cations and anions. Bubbling carbon dioxide through the solutions reduced significantly the absorption of all nutrient elements tested, by all three experimental plants. Bubbling air through the solution, in general, increased the absorption of the nutrient elements over the control plants, but the increment was much less prominent and consistent than the reduction caused by the carbon dioxide. Bubbling commercial nitrogen through the solutions in a single experiment with wheat did not affect the absorption of cations, but showed some depression on the absorption of phosphate. Covering the solution with a thin layer of paraffin oil caused no great reduction in the absorption of Ca, Mg, and P by maize plants when compared to the controls, but the depressing effect on K and N absorption was significant. Among the various elements, K absorption was most affected by carbon dioxide, loss of K to the solution being obtained in many jars.

Adjusting the acidity of solutions to pH 4.0 produced a reduction in absorption of total salt including NO_3 by rice plants. The absorption, however, was still significantly higher than in plants treated with carbon dioxide.

The results of these experiments support the conclusion that high concentration of carbon dioxide has a specific narcotic effect upon root

TABLE 2

POTASSIUM ABSORPTION UNDER DIFFERENT TREATMENTS. CK. IN MG. (EQUALS 100 PER CENT); OTHER TREATMENTS IN PERCENTAGE OF CK.

Crop	Experiment	Ck. (Mg.)	Aerated %	Nitrogen %	Oiled %	Plus CO_2 %
Wheat	I	7.4	256.7			−29.7*
	II	24.0	115.8	106.3		33.7
Maize	I	33.1	129.3		52.9	−23.6*
	II	31.1	166.9		50.8	1.6
Average %		100.0	167.2			−4.5*

* The negative percentages resulted from the release of K by the plants treated with CO_2.

TABLE 3

CALCIUM ABSORPTION UNDER DIFFERENT TREATMENTS. CK. IN MG. (EQUALS 100 PER CENT); OTHER TREATMENTS IN PERCENTAGE OF CK.

Crop	Experiment	Ck. (Mg.)	Aerated %	Nitrogen %	Oiled %	Plus CO_2 %
Wheat	I	3.1	45.2			0.6
	II	10.3	95.1	102.9		79.6
Maize	I	8.9	120.2		100.0	57.3
	II	13.1	156.4		119.1	74.0
Average %		100.0	104.2			52.9

TABLE 4

MAGNESIUM ABSORPTION UNDER DIFFERENT TREATMENTS. CK. IN MG. (EQUALS 100 PER CENT); OTHER TREATMENTS IN PERCENTAGE OF CK.

Crop	Experiment	Ck. Mg.	Aerated %	Nitrogen %	Oiled %	Plus CO_2 %
Wheat	II	7.0	105.7	104.3		55.7
Maize	I	4.1	153.6		100.0	65.8
	II	7.7	115.6		87.0	70.1
Average %		100.0	125.0			63.9

TABLE 5

NITROGEN ABSORPTION UNDER DIFFERENT TREATMENTS. CK. IN MG. (EQUALS 100 PER CENT); OTHER TREATMENTS IN PERCENTAGE OF CK.

Crop	Experiment	Ck. Mg.	Aerated %	Nitrogen %	Oiled %	Plus CO_2 %
Wheat	I	11.1	161.3			18.9
	II	18.0	97.8	95.0		83.9
Maize	I	10.2	94.1		53.9	36.3
	II	43.9	100.7		94.1	36.7
Rice	Acid+	5.1	107.8			11.8
	Acid−	8.0	143.7			11.2
Average %		100.0	117.6			27.0

TABLE 6

PHOSPHORUS ABSORPTION UNDER DIFFERENT TREATMENTS. CK. IN MG. (EQUALS 100 PER CENT); OTHER TREATMENTS IN PERCENTAGE OF CK.

Crop	Experiment	Ck. Mg.	Aerated %	Nitrogen %	Oiled %	Plus CO_2 %
Wheat	II	2.5	104.0	84.0		64.0
Maize	I	1.5	278.3		66.7	46.7
	II	4.0	207.5		115.0	22.5
Average %		100.0	196.6			44.4

protoplasm which decreases its permeability to water and minerals and apparently deprives the root cells of their ability to absorb ions against a concentration gradient. The important evidences are:

1. Carbon dioxide reduced the absorption of water, a nonelectrolyte, cations, and anions by all three experimental plants.

2. Nitrogen, which also served to sweep out the air from the solutions, failed, in a single experiment with wheat, to reduce the water absorption and cation absorption.

3. Adjusting the pH of control solutions to the value of those treated with carbon dioxide failed to reduce the water absorption. Although it caused a reduction in the absorption of salts, the amounts absorbed were still significantly higher than in plants treated with carbon dioxide.

I. OXIDATIVE DEGRADATION OF CELLULOSE-ACETATE RAYON
II. THERMAL DEGRADATION OF SOME CELLULOSIC TEXTILES BY STEAM[1]

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I. OXIDATIVE DEGRADATION OF CELLULOSE-ACETATE RAYON

Because references in the literature regarding oxidation of cellulose-acetate rayon are few and contradictory, a study has been made of the oxidizing bleaches, aqueous potassium permanganate, acidic potassium permanganate (0.05 *M* as to sulfuric acid), sodium peroxyborate (0.3 per cent as to soap), neutral calcium hypochlorite, and sodium *N*-chloro-*p*-toluenesulfonamide, on an undyed cellulose-acetate rayon taffeta. Degradation has been followed through changes in acetyl, copper number, wet strength, and weight. These changes have been compared with those observed in unbleached cotton cellulose and regenerated-cellulose rayon when oxidized similarly.

OXIDATION WITH POTASSIUM PERMANGANATE

Acetyl of cellulose-acetate rayon oxidized with either aqueous or acidic potassium permanganate showed an apparent increase, probably because carboxyl groups produced during oxidation reacted with the sodium hydroxide used in saponification for determination of acetyl, and were thus reported as acetyl.

In 4 hours at 40° C., 0.033 *M* aqueous permanganate caused the copper number of cotton cellulose, regenerated-cellulose rayon, and cellulose-acetate rayon to rise 3.56, 6.55, and 0.93, respectively, while in acidic solution the increments were 4.73, 7.69, and greater than 9, respectively. Loss of wet strength in aqueous permanganate under these conditions was 69 per cent for cotton cellulose, complete for regenerated-cellulose rayon, and 43 per cent for cellulose-acetate rayon; in acidic solution the loss was 83 per cent for cotton cellulose and complete loss for regenerated-cellulose rayon and cellulose-acetate rayon. Loss of weight was negligible for all textiles studied in aqueous permanganate, but in acidic bath cotton cellulose lost 3.1 per cent, regenerated-cellulose rayon 9.7 per cent, and cellulose-acetate rayon 45.3 per cent of its weight.

These results indicate that for cotton cellulose and regenerated-cellulose rayon the action of aqueous and acidic potassium permanganate is similar; in most cases effect of temperature is greater than effect of pH. For cellulose-acetate rayon, however, the effect of pH is greater than effect of temperature; this textile, while more resistant to oxidative degradation by aqueous permanganate than cotton cellulose or regenerated-cellulose rayon, is more vigorously attacked in acidic solution.

[1] Doctoral thesis number 737, submitted December 15, 1943.

Oxidation With Sodium Peroxyborate

Acetyl of cellulose-acetate rayon was decreased to a negligible extent by 0.1922 *N* sodium peroxyborate in 8 hours at 40° C. but was reduced by 30 per cent in 2 hours at 100° C. Loss in acetyl was shown to be a linear function of concentration of oxidant, probably because of alkali released during oxidation.

The effect of sodium peroxyborate on unbleached cotton cellulose at 40° C. for 8 hours and at 100° C. for 2 hours is approximately equal and negligible for concentrations up to 0.1922 *N*. Regenerated-cellulose rayon also was attacked equally at both these temperatures but much more considerably than cotton cellulose. Rise in copper number for regenerated-cellulose rayon was 1.84, loss in wet strength 57 per cent, and loss in weight only 0.7 per cent at 0.1922 *N* concentration of oxidant.

The effect of sodium peroxyborate in concentrations up to 0.1922 *N* on cellulose-acetate rayon for 8 hours at 40° C. is negligible. At 100° C. for 2 hours, however, with this concentration, loss in wet strength was approximately 40 per cent, of which only 10 per cent may be ascribed to deacetylation, the remainder to oxidation. Copper number showed progressive linear increase with saponification. Loss in weight was approximately 3 per cent greater than that caused by loss of acetyl, and this, too, was attributed to loss by oxidation.

Oxidation With Calcium Hypochlorite

During treatment with 0.1 *N* neutral calcium hypochlorite, cellulose-acetate rayon was attacked to no noticeable degree until it was oxidized for 4 hours at 40° C. when it lost but 26 per cent of its wet strength, in contrast to cotton cellulose which lost 64 per cent and regenerated-cellulose rayon which was left with no measurable wet strength. In 4 hours at 25° C. cotton cellulose lost 18 per cent and regenerated-cellulose rayon 96 per cent of its wet strength, whereas the wet strength of cellulose-acetate rayon similarly oxidized was unimpaired. Copper number and loss of weight in each case reflected loss in wet strength. No significant change in acetyl of cellulose-acetate rayon was observed in oxidation with calcium hypochlorite.

Oxidation With Sodium-*N*-chloro-*p*-toluenesulfonamide

Neither cotton cellulose nor cellulose-acetate rayon showed any loss in wet strength when oxidized 4 hours at 40° C. in concentrations up to 0.3 *N*, although regenerated-cellulose rayon lost 22 per cent of its wet strength. At 100° C. regenerated-cellulose rayon retained no measurable wet strength even in concentration as low as 0.1 normal. Cotton cellulose retained 77 per cent of its wet strength after 4 hours at 100° C. in 0.3 *N* bath, although cellulose-acetate rayon lost 56 per cent in 0.1 *N* and was disintegrated in 0.2 *N* bath. Copper number and weight of cotton cellulose and regenerated-cellulose reflected these changes. Copper number of cellulose-acetate rayon decreased, although change in acetyl was neg-

ligible, and weight was increased. The increment in weight was thought caused by absorption of the oxidant or its product, *p*-toluenesulfonamide, inasmuch as the textile was yellowed and its depth of color increased with concentration of oxidant.

These results indicate that under mild conditions of oxidation, cellulose-acetate rayon is more resistant than either cotton cellulose or regenerated-cellulose rayon, but that when oxidative action becomes more drastic, it is attacked to a greater extent.

II. THERMAL DEGRADATION OF SOME CELLULOSIC TEXTILES BY STEAM

The action of steam in 1.5 hours at 10, 30, and 60 pounds gauge pressure (115.0°, 134.5°, and 153.0° C., respectively) on unbleached and bleached cotton cellulose, regenerated-cellulose rayon, and cellulose-acetate rayon has been followed by acetyl, copper number, wet strength, and weight. Unbleached cotton lost 75 per cent of its wet strength in 1.5 hours at 153° C. in contrast to bleached cotton cellulose which lost but 47 per cent. Regenerated-cellulose rayon was shown surprisingly resistant to heat, with loss of but 23 per cent of its wet strength. Cellulose-acetate rayon retained only 32 per cent of its wet strengh at 153° C.; this suggested that the loss of strength was brought about by the acetic acid released upon hydrolysis, although loss of acetyl was slight. Loss of weight was negligible for each textile except unbleached cotton cellulose, which lost 4.6 per cent at 153° C. Depth of color of each textile increased as the temperature increased; unbleached cellulose steamed at the highest temperature was of a deep brown color. Starch size provided protection against the action of steam, inasmuch as unbleached and bleached cotton cellulose desized with *Taka-Diastase* were of lower wet strength than when sized.

When increment in copper number was plotted against decrease in wet strength, a line of the general equation $y = mx + b$ resulted within experimental error; m was shown to be different for each textile.

THE PASTEURIZATION OF LIQUID WHOLE EGG[1]

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The rapid growth of the liquid whole egg industry during the last 20 years has led to the development of a serious bacteriological problem.

Liquid whole egg is the raw material used for the preparation of frozen and dehydrated egg. It is prepared in egg-breaking plants by breaking the shell-egg, collecting the contents, and thoroughly mixing the product. During the process some precautions are maintained to control, as much as possible, the microbial contamination of the melange. However, in spite of these controls the production of a commercial whole egg containing fewer than 100,000 micro-organisms per gram is comparatively rare. Liquid whole egg containing more than 100,000 micro-organisms per gram deteriorates very rapidly when stored at temperatures above 10° C.

Because of the prevalence of high count egg products in commercial channels, they are constantly under surveillance by public health officials. The presence of pathogenic micro-organisms in such products causing intestinal and other infections in human beings has been suggested from time to time. The possibility of *Salmonella* and *Mycobacterium* infections from egg products is not to be minimized.

These facts led many workers to suggest the possibility of pasteurizing liquid whole egg as a means of improving its keeping qualities and making it "safe." However, it has been reported repeatedly that egg products could not be pasteurized because of the rapid denaturation of the proteins at temperatures which were bactericidal.

This investigation was concerned with studying: the tentative maximum times and temperatures to which egg-melange could be subjected without markedly affecting the proteins, the rate of bacterial destruction by heat of micro-organisms present in egg-melange and the possibility of commercially applying pasteurization to whole egg.

Determinations on the effect of heat on the denaturation of egg-proteins, as measured by the increase in relative viscosity, were made between 56–68°C. Arbitrary standard for tentative allowable denaturation was fixed at 50 per cent increase in relative viscosity. The studies indicated that the rate of denaturation was 250 times more rapid at 68°C. than at 56°C.

Because of its extreme heat resistance, *E. coli* (culture H_1) was used for most of the cell destruction tests. A much less resistant strain of the same organism [P_{19} isolated from egg-melange which had been heated for 1 hour at 136°F. (commercial practice in one plant)] was also studied.

Over 99 per cent cell-destruction of culture H_1 was achieved at the

[1] Doctoral thesis number 734, submitted December 10, 1943.

temperatures studied (56, 59, 62.5 and 66°C.) within the limits imposed by the denaturation of the proteins in the egg-melange. When culture P_{19} was used the period of exposure to produce 99 per cent bacterial destruction at 62.5°C. was less than 1 minute, whereas culture H_1 required 6 minutes.

The age of the eggs used in preparing the melange was shown to have a definite effect on the thermal resistance of bacteria suspended in it.

Bacteria were more easily destroyed by heat in melange having a pH of 7.4–7.6 as compared to that of 6.4–6.8. This effect probably accounts for the differences between the results with fresh and aged eggs. A short growth period (4–5 hours) of culture H_1 in the melange markedly reduced its thermal resistance.

The comparative pasteurizing effect was studied with culture H_1 inoculated simultaneously into egg-melange and raw milk. In every instance it was demonstrated that the bacteria could be more easily destroyed in egg-melange than in milk. The time necessary to produce 99 per cent cell-destruction was 7 minutes in egg-melange and 17.5 minutes in milk. This difference was largely due to pH (pH of milk 6.5, that of egg-melange 7.6). When the pH of egg-melange was adjusted to 6.5, the thermal resistance of the culture in milk and eggs was approximately the same.

The thermal resistance during pasteurization of a strain in a mixture of micro-organisms was the same as when it was tested separately. Over 99 per cent cell-destruction of the mixed organisms was obtained in less than 6 minutes at 62.5°C.

Of the six cultures isolated from liquid whole egg, those of the genus *Actinomyces* were found to be the most resistant.

One commercial trial made with a high-temperature-short-time milk pasteurizer (Creamery Package unit) indicated that egg-melange could be pasteurized effectively (99 per cent destruction) by heating the melange for 32.5 seconds at 67°C. The few micro-organisms which resisted this treatment were identified as members of the species *Leuconostoc citrovorum*, an organism commonly found in milk.

Cake-baking and custard-making tests (using the commercially pasteurized melange) indicated no deleterious effect as a result of this heat treatment.

Other results reported indicate that probably other types of milk pasteurizing equipment could be used successfully if they possess sufficiently sensitive controls.

THE MECHANISM OF FORMATION OF ACETYLMETHYLCARBINOL BY ACTIVE ENZYME PREPARATIONS[1]

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Acetylmethylcarbinol as the precursor to diacetyl is responsible, in part, for the development of desirable flavors in many foods. This fact is especially true of high quality dairy products and bakery goods.

Investigators are not agreed on the origin of acetylmethylcarbinol. Considerable evidence has been presented to support the view that acetaldehyde is an intermediate in the formation of the carbinol from glucose, whereas other investigators have reported the formation of the carbinol from pyruvate.

Silverman and Werkman (1941) were unable to show an increased yield of acetylmethylcarbinol from pyruvic acid when acetaldehyde was added to their cell-free preparation of *Aerobacter*. Green *et al.* (1942) have shown that the addition of acetaldehyde to pyruvate in the presence of a yeast juice gives an increase in the yield of acetylmethylcarbinol.

Active bacterial enzyme preparations were obtained from *Aerobacter aerogenes* by a method developed in this laboratory. Wiggert *et al.* (1940) obtained an active juice from bacteria by mixing a bacterial paste with very finely powdered glass and grinding the mixture in a mortar. Later, a mechanical grinding procedure superseded the earlier method. The bacterial enzyme preparations were very active on glucose or sodium pyruvate when tested on the Barcroft-Warburg respirometer under an atmosphere of nitrogen. The activity was measured by the CO_2 produced and by the amount of acetylmethylcarbinol formed.

The yeast juices were prepared from dried yeast. The yeast was autolyzed and the active principle was precipitated by $(NH_4)_2SO_4$ and redissolved in phosphate solution according to the method of Green *et al.* (1942).

The yeast juices were very active on glucose and sodium pyruvate as shown by manometric determinations. Considerable amounts of acetylmethylcarbinol were formed from pyruvate.

Freezing the yeast juice decreased the rate of formation and the total amount of CO_2 produced from sodium pyruvate.

Increasing the concentration of substrate (sodium pyruvate) increased the amount of CO_2 produced. The amount of acetylmethylcarbinol was increased also. The increases in CO_2 and acetylmethylcarbinol were not proportional to the increase in substrate added.

The addition of acetaldehyde to the yeast juice increased the amount of CO_2 and acetylmethylcarbinol formed.

[1] Doctoral thesis number 747, submitted June 6, 1944.

The animal juice was obtained from minced pig heart by precipitation with acetic acid and resuspended in phosphate buffer. The activity of this juice, as determined manometrically, was not high because only small amounts of CO_2 were formed from pyruvate. The amount of acetylmethylcarbinol formed, however, was much greater than with the bacterial or yeast juices.

The mechanism of formation of acetylmethylcarbinol was studied using isotopic carbon (C^{13}) as the tracer. Acetaldehyde containing C^{13} in both positions was employed. Fermentations were conducted with the various enzyme preparations using sodium pyruvate and the isotopic acetaldehyde as the substrates.

The acetylmethylcarbinol formed by the bacterial juice contained the normal complement of C^{13}. This finding confirmed the investigations of Silverman and Werkman (1941) that added acetaldehyde did not enter into the carbinol formation by their cell-free juice.

The acetylmethylcarbinol formed in the yeast juice fermentations contained a considerable increase in the C^{13}. The only source of enriched C^{13} was the acetaldehyde. These data indicate that yeast juice can utilize synthetic aldehyde in the formation of the carbinol.

The pig heart juice formed acetylmethylcarbinol from aldehyde alone with no production of CO_2. The addition of pyruvate did not increase the carbinol production.

The isotopic carbon composition of the aceylmethylcarbinol formed by the yeast juice containing the increased C^{13} content was determined. Each carbon group of the carbinol was split out of the molecule and analyzed for C^{13}.

The methyl group, adjacent to the keto carbon, was split off by the iodoform reaction leaving lactic acid. The lactic acid molecule was oxidized to CO_2 and acetaldehyde. The CO_2 contained the carbonyl carbon of the acetylmethylcarbinol molecule. The methyl group was removed from the aldehyde by the iodoform reaction. The formic acid formed by the above reaction contained the carbon from the original carbinol group of the acetylmethylcarbinol.

In other experiments the acetylmethylcarbinol was split into acetic acid and acetaldehyde by the KIO_4 oxidation.

Data obtained indicate that each carbon of the C^{13} acetylmethylcarbinol was enriched with C^{13} but not to the same degree. The carbonyl end of the molecule contained the smaller amount of heavy carbon. The carbinol end contained a greater percentage of heavy carbon than the whole original acetylmethylcarbinol molecule. These data indicate a greater fixation of the synthetic acetaldehyde in the carbinol end of the molecule.

Many dried yeast preparations were investigated to find an active juice, with little success.

An apparatus is described for collecting and weighing the CO_2 directly from the oxidation reaction. This bulb eliminates the barium salt step formerly used with the mass spectrometer analysis.

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MEAT IN NUTRITION. XV. CERTAIN CHARACTERISTICS OF GESTATIONAL PERFORMANCE IN ALBINO RATS FED A DIET CONTAINING DRIED AUTOCLAVED PORK MUSCLE[1]

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Reports from the Nutrition Laboratory of the Foods and Nutrition Department at the Iowa State College have shown that feeding rats a supposedly adequate diet containing dried autoclaved pork muscle consistently produces both partial and complete gestational failures. In partial gestational failure, the most striking abnormality is a high mortality of the young during the first 4 days of life; in the complete, an acute disturbance at the time of parturition that results in the death of both mother and feti. The chief purpose of the present study was to establish the syndrome characteristic of each type of gestational failure. In addition, the effect of adding three supplements, lipocaic, fresh liver, and liver extract, to the basal pork diet was investigated.

The 235 animals used in the experiment were divided into three experimental groups, *i.e.*, (1) the control group of rats fed the stock colony diet known as Steenbock V which has never been known to produce complete gestational failure, (2) the group of rats receiving the experimental (basal pork) ration, and (3) the group of animals receiving the experimental ration supplemented by fresh liver, lipocaic, or liver extract. To determine the influence of pregnancy *per se,* pregnant and virgin females were maintained on each diet. The experimental groups of pregnant animals were further subdivided on the basis of the diet of the males used for mating.

The females in the pregnant series were allowed to bear and rear one litter each. They were then killed 21.5 days following the initiation of the second pregnancy. The general physical condition of the animals and the appearance of certain organs were described at the end of the experiment. The liver, kidney, heart, spleen, feti, placentae, and in some cases the pancreas were removed at autopsy. The virgin animals in each group were killed when they had received the diet the same number of days as the pregnant animals in that group.

In the first part of the study the gestational performance of animals in the three experimental groups was evaluated. Data collected on the progression of the first gestation period and the condition and vitality of the first litter were used in this analysis. In addition, data pertaining to the second pregnancy, obtained by observation on the progression of gestation, and the condition of the uterine contents at autopsy were studied. The following observations were made:

1. The second pregnancy was a better measure of the effect of diet upon gestational performance than was the first pregnancy,

[1] Doctoral thesis number 524, submitted June 7, 1939.

2. The feeding of the pork-containing diet resulted in poorer gestational performance than that noted in animals fed the adequate control ration,

3. Gestational failures, both partial and complete, were more numerous in females mated with males also receiving the basal pork-containing diet, than when males from the stock colony were used for mating,

4. Fresh liver was the only supplement added to the basal pork diet that prevented the appearance of the pregnancy disorder, and

5. The feeding of the sample of lipocaic used markedly increased the occurrence of resorptions, as many as 68 per cent of the feti being lost in rats fed 500 mg. of the supplement daily.

The second part of the investigation consisted of a study of the pathological changes associated with complete gestational failure. Such failure occurred in about 35 per cent of the pregnant animals receiving the pork ration. Changes in general physical condition of the animals, gain in body weight during pregnancy, water consumption in pregnancy, fat content of the liver, weight and moisture content of organs, and histology of organs, feti, and placentae were considered.

The general physical condition of the animals both in regard to external appearance and condition of certain visceral organs was rated subjectively. In addition, the rectal temperature of the pregnant animals was taken. The gains made in body weight during gestation were studied, pairing experimental animals with normal females from the stock colony matched in respect to body weight at the initiation of pregnancy and number and weight of feti. The amount of water consumed by the pregnant animals was measured twice daily from the twelfth day of pregnancy until parturition. The average weight and moisture content of the liver, kidney, spleen, heart, and mammae were determined. In addition, analyses were made of the fat content of the liver.[2] Histological sections were prepared of the liver, kidney, heart, spleen, and pancreas, as well as of the feti and placentae. A standard method using Zenker's solution for the fixative and haematoxylin and ethyl eosin as stains was followed in the preparation of the sections.

The feeding of the pork diet to the virgin animals increased the relative quantity of fat in the liver and induced cellular changes in the liver and the kidney. These differences were accentuated by pregnancy. In the control group fed the stock colony diet, the virgin animals were normal and pregnancy produced only slight cellular changes.

Between the apparently healthy pregnant animals fed the various pork-containing diets and the normal control group, the only consistent differences noted were in the liver. The livers from the rats receiving pork muscle were higher in fat and lower in moisture than those of the normal control animals. In addition, an increase in cloudy swelling in the hepatic cells was observed upon histological examination of the sections prepared from livers of pork-fed rats. Some degenerative changes were also noted in the kidneys of these rats.

[2] These data were included through the courtesy of Dr. Ethelwyn Wilcox.

Deviations from normal were marked in the ten animals that died due to the pregnancy disorder. In general, the symptoms may be described as follows:

1. The sick animals made excessive gains in body weight during the last day of pregnancy; the relative moisture contents of the liver, kidney and spleen of these animals as well as changes in water consumption suggested that the large gains in body weight were due to a disturbance in water balance;

2. The liver was yellow in color, large in size, and friable in consistency, the quantity of fat was abnormally high, and the hepatic cells showed marked fat degeneration and infiltration;

3. The kidneys were swollen and gorged with blood;

4. The feti were well-developed, but invariably dead and all showed hemolysis of fetal blood and thrombi in the umbilical veins, and

5. The placentae were anemic.

Finally, an attempt was made to evaluate the significance of the findings. In so doing, similarities between the pregnancy disorder described above, eclampsia in women, and disturbances of gestation reported in rabbits and sheep were indicated. A theory was developed that might explain the train of events observed in the pregnancy disorder; also, the changes found in partial gestational failure were correlated with those observed in complete gestational failure.

The following general conclusions may be drawn from the data:

1. Both the partial and complete gestational failures observed in animals fed the basal pork-containing ration may be prevented by dietary means and hence are due to a lack of some factor or factors in the basal ration,

2. The fundamental disorder of dietary origin is aggravated to a serious level by the imposition of pregnancy,

3. A specific syndrome is characteristic of the pregnancy disorder,

4. The pregnancy disorder occurring in gravid rats fed the basal pork ration is strikingly similar to eclampsia in women and seems typical of a general metabolic disturbance.

THE USE OF SOME AGRICULTURAL PRODUCTS AS RAW MATERIALS IN THE PLASTIC INDUSTRY [1]

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Plastics have developed into a major industry in the last 30 years. The limiting factor of this industry at the present is the cost of raw materials. The plentifulness and cheapness of agricultural products, and particularly certain by-products, make them inviting as possible plastics raw materials. This thesis is a study of the production of low cost plastics from agricultural materials.

FURFURAL-SOYBEAN MEAL PLASTICS

Since it is known that a protein and an aldehyde react to form a condensation product, it would be expected that such a reaction would take place between soybean meal, which is high in vegetable protein, and either formaldehyde or furfural.

In the early studies, furfural, phenol, and ammonia were mixed together in a beaker heated in a bath of boiling water. Soybean meal and lime were added and the pasty mass stirred and heated from 2 to 5 hours, after which it was dried for several days at 60°C. The now brittle material was pulverized and mixed with approximately equal weight of filler (wood flour or asbestos) and 4 per cent hexamethylene tetramine. This mixture was formed into test buttons in a heated mold under hydraulic pressure. In later studies using larger amounts of materials, the cooking was done in a small steel cooker with a power-driven stirrer.

As the result of studies on the effect of such variables as the relative amounts of constituents, cooking time, and method of adding and mixing ingredients, the optimum product was found to be produced as follows: Thirty parts by weight of furfural, 24 parts of phenol, and 4 parts ammonium hydroxide were refluxed for 1 hour. To this were added 36 parts soybean meal and 3 parts lime. The mixture was then heated with constant mixing for 4.5 hours at 110° to 120°C. It was then dried and ground to pass a 60-mesh screen. Forty parts of this ground resin were then mixed with 60 parts of asbestos filler and 6 parts hexamethylene tetramine. The resulting molding powder was molded at 200°C. and 1,800 pounds per square inch for 3.5 minutes, followed by cooling under pressure in the mold for 2 minutes. Test buttons made in this manner withstood a drop test of a 5-pound weight from a height of 24 inches, and had a water absorption of 0.55 per cent in 24 hours, thus comparing favorably with similar types of commercial plastics. When the hexamethylene tetramine was reduced to 2 per cent, the water absorption dropped to 0.14 per cent and the strength to 14 inches. There is also experimental evidence to

[1] Doctoral thesis number 499, submitted December 17, 1938.

show that the substitution of water extracted soybean meal produces a plastic with a lower water absorption than unextracted meal. Plastics using wood flour as a filler had both greater strength and greater moisture absorption than those using asbestos.

Other workers in this laboratory had developed a plastic from corncobs, cresol, and sulfuric acid, which was characterized by low water absorption, good moldability, and excellent appearance. The strength of this plastic was somewhat lower than the soybean product. The two plastic resins were blended together in varying proportions, mixed with filler, and molded. The optimum mixture was found to be 11 parts of the corncob-cresol resin, 33 parts soybean resin, 66 parts asbestos filler, and 6 parts hexamethylene tetramine. Test pieces molded from this mixture failed to break under a drop test of 24 inches (the limit of the testing machine) and had a moisture absorption of 0.33 per cent, thus combining the good characteristics of the two products.

The corncob-cresol plastic used in this mixture was made as follows: One hundred ninety-one grams cresol, 39 grams sulfuric acid (1 part concentrated acid to 1 part water), and 130 grams corncobs were refluxed at 110°C. for 2 hours followed by heating without the condenser to 250°C.

The raw material cost of the furfural-soybean phenol resin is 10 cents per pound. The molding powder with wood flour filler is 7 cents a pound, and with asbestos filler is 6 cents a pound. The material cost for the molding powder composed of the two types of resins with asbestos filler varies from 3.5 to 6 cents a pound depending on the quality of the product produced.

PLASTICS FROM HYDROLYZED AGRICULTURAL BY-PRODUCT MATERIALS

The agricultural by-products furnish the largest supply of organic material in the world. Most of these materials are hydrolyzed by low acid concentrations to produce xylose. The production of xylose from cornstalks was studied as a separate problem, and the hydrolyzed residue remaining from this study was used as a raw material for plastics. The cornstalks were cooked in water at 15 pounds per square inch steam pressure for 2 hours, washed with 0.25 *N* sulfuric acid, cooked at 70 pounds steam pressure with 0.2 *N* sulfuric acid for 2 hours, and washed with water. The hydrolyzed residue was ground to pass a 60-mesh screen and then treated with furfural, aniline, and other reagents. The best product was produced from 67 per cent hydrolyzed cornstalks, 15 per cent furfural, 15 per cent aniline, and 3 per cent lime cooked at 115° for ½ hour. This material had a good appearance, a strength of 23 inches in the drop test machine, and a water absorption of 1.01 per cent. Hydrofurfuramide was substituted for furfural with equally good results. Unhydrolyzed stalks did not produce a good plastic. Excellent products were also made from lignin, furfural, urea, and aniline.

The hydrolyzed cornstalk-furfural-aniline-lime plastic molding pow-

der without filler had a raw material cost of $0.028 per pound. The addidition of filler would reduce this cost.

SUMMARY

Satisfactory plastic molding compounds have been produced from mixtures of soybean meal, furfural, and phenol, and of this material with a molding compound from corncobs, cresol, and sulfuric acid. The raw material cost is about 6 cents a pound.

A good plastic was also produced from hydrolyzed cornstalks, furfural, aniline, and lime at a raw material cost of less than 3 cents a pound.

PATHOGENICITY ON AVENA AND GROWTH RESPONSE OF *PSEUDOMONAS CORONAFACIENS* (ELLIOTT) STAPP[1]

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In a study conducted in 1940, 1941, and 1942 *Pseudomonas coronafaciens* (Elliott) Stapp, the causal organism of halo blight, was observed to attack species and varieties of Avena from the time the seed coat ruptured until the plant was mature. Symptoms were observed on the coleoptiles, culms, leaf sheaths, leaves, and glumes. The plumule within some infected seed was found to be entirely destroyed, and the radicle remained underdeveloped. In some cases the entire seed, excepting the lemma and palea, was rotted to such an extent that if pressure were applied, a yellowish-white viscous material was extruded. Varying degrees of severity of plumule necrosis were observed ranging from seedlings on which the plumule could not be observed to those in which the above ground parts attained almost full development after emergence. On leaves, the part of the plant most commonly attacked, symptoms appeared first as tiny water-soaked spots 1 mm. or less in diameter which became the centers of lesions showing yellowed, haloed areas, rapidly becoming browned and confluent or of concentrically ringed appearance. The effect of variety of host on symptoms was evident and consisted essentially of variation in color, size and number of lesions, and definiteness of concentrically ringed or confluent appearance. Lesions on leaf sheaths observed were elongate, yellowed, confluent areas commonly lacking the concentrically ringed appearance. Lesions on culms and glumes were observed infrequently.

Numerous isolations were made in 1940, 1941, and 1942 from oat leaf lesions of suspected bacterial origin. The lesions showed considerable variations in size, shape, color, and amount of halo. There was some difference in lesion type between host varieties. Isolates from linear lesions with observable exudate were designated as *Ps. striafaciens* and did not differ in cultural reactions from isolates of *Ps. coronafaciens*. Isolates from *Bromus inermis* identified as *Ps. coronafaciens* var. *atropurpureum* were similar in cultural reactions to isolates of *Ps. coronafaciens*, with the exceptions of the production of fluorescence in beef-peptone broth and their slightly more rapid growth. The isolates from brome were pathogenic on oats.

The isolates from oats and brome grass were observed on and in various selected media to determine whether difference in reaction existed among isolates from different hosts and types of lesions and to provide a basis for comparison with the characteristics of isolates described by previous workers. The characters observed were gross morphology, tempera-

[1] Doctoral thesis number 710, submitted March 15, 1943.

ture relations, gelatin liquefaction, nitrate reduction, hydrogen sulfide production, ammonia production, reaction in litmus milk, "Imvic" reaction, carbohydrate utilization, and starch hydrolysis. Cultural studies of 9 isolates of *Ps. coronafaciens* obtained in 1940, 18 isolates obtained in 1941, and 27 isolates obtained in 1942 showed that the organism present on oats in Iowa in these 3 years was essentially like the causal organism previously described by Elliott.

There was some evidence of the occurrence of cultural strains among the cultures of *Ps. coronafaciens* in carbohydrate utilization—rhamnose, d-galactose, and sucrose; but clearly defined cultural strains could not be established. Isolates of *Ps. coronafaciens* var. *atropurpurem* obtained from *Bromus inermis* and isolates of *Ps. striafaciens* obtained from oat plants were culturally similar to isolates of *Ps. coronafaciens*.

Isolates were tested for pathogenicity by atomizing water suspensions of bacteria on uninjured leaves of seedling oat plants, by hypodermic injection of the suspension into the culms of juvenile plants, and by seed infection. Seed infection was obtained by soaking oat seeds, from which the hulls had been removed, in a water suspension of the bacterial cells, or by placing oat seeds with hulls on in the suspension and holding them under a partial vacuum equal to 22 to 25 inches of mercury for 30 minutes. It was necessary to remove the hulls or place the bacteria under the hulls by use of a partial vacuum before consistent and heavy seed infection could be obtained. Oat varieties inoculated in this manner showed striking increases in pre-emergence killing and in severity of disease on the surviving seedlings. Certain varieties and selections were much more susceptible than others. Varietal response, using this method of inoculation, was in closer agreement with field observations than when plants were sprayed with a suspension of bacteria or inoculated by means of hypodermic injection. The degree of susceptibility of the varieties changed with the conditions under which the test was conducted. The behavior most like the field leaf reaction occurred in the tests at 70°F. At a lower temperature (50°F.) separation of the disease injury into two categories was evident, particularly in the tests with hulled seed. The severity of disease on the leaves agreed in general with the field leaf reaction studies. The second effect, that of pre-emergent killing, seemed to be relatively unrelated to the severity of leaf symptoms. It seems that utilization of this seed infestation method is of real value in evaluation of varietal response in Avena.

The data presented indicate that *Ps. coronafaciens* is a more prevalent and destructive pathogen on oats in Iowa than formerly was realized. It is evident that seedling injury and killing may play a considerable part in the reduction of oat stands and in providing sources of inoculum for subsequent spread. The range of leaf symptoms studied allows the inclusions of several types of leaf injury heretofore not definitely attributed to this organism.

Studies of field reaction of varieties and selections of oats in Iowa revealed striking and consistent differences in susceptibility to *Ps. corona-*

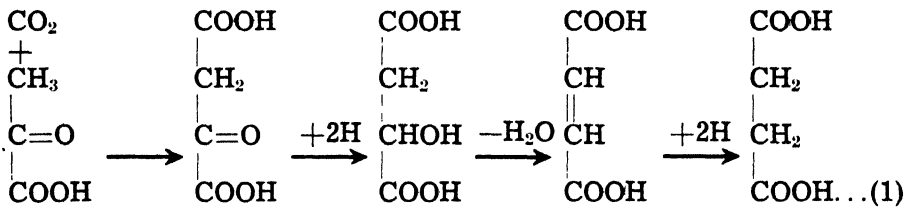
faciens in the 3 years of study. Such examination showed adequate sources of field resistance. Boone, Marion, Hancock, Erban, Anthony, Mutica Ukraina, Gopher, and Landhafer, and selections from crosses involving them as parents, were in general susceptible to halo blight. Victoria x Richland selections were for the most part intermediate in susceptibility although certain selections, such as Boone, were highly susceptible under field conditions. Selections involving Bond as one parent were, in most cases, comparatively free from halo blight. D-69 x Bond selections, as a group, were outstanding for resistance.

PYRUVATE DISSIMILATION BY BACTERIAL ENZYME PREPARATIONS [1]

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Pyruvate is one of the most important intermediary substances formed during metabolism. It participates in a variety of reactions and is apparently the cardinal intermediary of carbohydrate, fat, and protein metabolism. Wood and Werkman (1940) postulated the carboxylation of pyruvic acid to oxalacetic acid and the conversion of the latter to malic, fumaric, and succinic acids:



...(1)

This series of reactions was proposed as the mechanism of heterotrophic utilization of carbon dioxide. The use of the isotopes of carbon has confirmed the occurrence and extended our knowledge of this reaction (*cf.* Werkman and Wood, 1942). In order to further elucidate the mechanism and possible physiological function of the fixation reaction, attempts have been made to utilize another tool, namely a cell-free enzyme preparation.

Bacterial enzyme preparations were obtained by grinding the cells with powdered pyrex glass, according to the method of Wiggert *et al.* (1942), and as further developed by workers in this laboratory. By varying the medium and conditions of growth, cells of *Escherichia coli* were obtained which, on being ground, yielded an enzyme preparation that was quite active anaerobically on pyruvate.

The optimal pH for the dissimilation of pyruvate by the enzyme preparation is 6.77 to 6.80. The preparation can be easily inactivated by heating at 55° to 57° for 5 minutes, and can be conveniently reduced to a powder by freezing and drying *in vacuo* with no immediate loss in activity. The enzymes formic dehydrogenase and hydrogenase are also present, and retain most of their activity even after several months in the dried state.

By subjecting the enzyme preparation to dialysis, it was determined that phosphate, manganese, cocarboxylase, protein, and an unknown substance are necessary for the action of the enzyme system responsible for the anaerobic dissimilation of pyruvic acid to acetic and formic acids.

The main path of pyruvate dissimilation by this preparation is that

[1] Doctoral thesis number 732, submitted November 8, 1943.

leading to acetic and formic acids, but small amounts of carbon dioxide and lactic and succinic acids are also formed.

In manometric experiments small amounts of carbon dioxide are fixed by the enzyme preparation with pyruvate and bicarbonate as substrates. In the presence of $NaHC^{13}O_3$, the fixed carbon dioxide was traced to the carboxyl groups of the succinic and lactic acids formed. Very little excess C^{13} was located in the formic acid, indicating that in the absence of hydrogenlyase, formic acid is not formed by a reduction of CO_2 as such but arises from the carboxyl group of pyruvic acid.

Carbon dioxide fixation is not the only mechanism of succinic acid formation by this enzyme system. On addition of $CH^3 \cdot C^{13}OOH$, succinic acid was isolated containing excess C^{13} exclusively in the carboxyl groups. Therefore, condensation of acetic acid, or its derivative with a 2-carbon or 3-carbon molecule, is another mechanism for the formation of succinic acid.

Evidence supporting the scheme for heterotrophic carbon dioxide utilization (1) has been presented by other workers. Krebs and Eggleston (1941) showed that succinate was formed from pyruvate, oxalacetate, malate, and fumarate by the propionic acid bacteria. Other investigators (Wood *et al.*, 1940, 1941, 1942; Nishina *et al.* ,1941), using the isotopes of carbon, located the fixed carbon dioxide in the carboxyl groups of the succinic fumaric and malic acids formed. Oxalacetate is the prime intermediate in the fixation reaction, but because of the instability and rapid dissimilation of oxalacetate, its direct formation from pyruvate and carbon dioxide has proved difficult. Krampitz *et al.* (1943), using an acetone preparation of *Micrococcus lysodeikticus*, could not demonstrate the formation of oxalacetate *via* direct carboxylation of pyruvate. However, employing the acetone preparation and the heavy carbon isotope, they did demonstrate that during decarboxylation of oxalacetate and carbon dioxide, some carboxylation occurred. This was the first direct evidence that oxalacetic acid or its derivative was a component of the fixation reaction, and that this reaction was reversible.

The enzyme preparation obtained from *E. coli* exhibits strong activity with fumarate and oxalacetate as acceptors of gaseous hydrogen. Oxalacetate is also rapidly decarboxylated. Manganese is necessary for the decarboxylation of oxalacetate, whereas cocarboxylase and inorganic phosphate are not. The specific protein nature of the enzyme concerned in this reaction was demonstrated.

The enzyme forms oxalacetate or a compound closely related to it from fumarate and malate and in smaller amounts from succinate, aerobically, thus demonstrating the reversibility of the reactions postulated above. No oxalacetate is formed from fumarate anaerobically. During the decarboxylation of oxalacetate to pyruvate and carbon dioxide by the enzyme in the presence of $NaHC^{13}O_3$, a carboxylation of pyruvate takes place, and an excess of C^{13} was located in the carboxyl group adjacent to the methylene carbon of the residual oxalacetate.

In attempts to demonstrate the carboxylation of pyruvate, no oxalacetate was detected under optimal conditions for carbon dioxide fixation

and succinic acid formation by the juice under an atmosphere of 10 per cent CO_2 in H_2. Under the same conditions with nitrogen substituted for hydrogen, definite tests were obtained for the formation of small amounts of oxalacetate or a compound very closely resembling it from pyruvate and carbon dioxide. The amounts of "oxalacetate" formed, although small, vary with the concentration of enzyme, pyruvate, and carbon dioxide.

Possible sources of energy for the carboxylation reaction are discussed.

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THE CONVERSION OF FERMENTATION PRODUCTS TO ELASTOMER INTERMEDIATES[1]

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INTRODUCTION

A fermentation product, ethyl alcohol, has long been considered the most useful single organic compound, because of the great multitude of its applications in the chemical industry. Ethyl alcohol has recently achieved further prominence by reason of the success encountered in converting the alcohol into butadiene-1,3, the "building block" of synthetic rubber. Many other valuable chemicals besides ethyl alcohol may be produced by fermentation, and the purpose of the work upon which this thesis was based was to investigate the possibility of extending the use of fermentation products, other than ethyl alcohol, for the formation of chemicals for the elastomer industry.

EXPERIMENTAL

BUTADIENE

The production of butadiene by dehydration of 2,3-butylene glycol was first investigated. A series of runs totaling almost 200 was made using a great variety of catalysts and varied reaction conditions, but yields of butadiene never exceeded 10 per cent of theoretical. The predominant reaction product in most of these runs was methyl ethyl ketone. Glycols generally yield carbonyl compounds upon dehydration, according to Ipatieff (1936), and 2,3-butylene glycol proved to be no exception to this rule.

Various esters of 2,3-butylene glycol give rise to butadiene upon pyrolysis. In particular the diacetate of 2,3-butylene glycol has been converted to butadiene in excellent yield on the pilot plant scale at the Northern Regional Research Laboratory. The possibility of converting other derivatives of butylene glycol, the monomethyl ether and the inner carbonate, to butadiene was next investigated.

The monomethyl ether of butylene glycol was prepared according to the directions of Chappell (1935). The inner carbonate had not previously been described. It was prepared by the action of phosgene upon 2,3-butylene glycol, and a continuous counter-current method of reaction was devised. Yields of 75 per cent of theoretical were realized. The compound was found to have the following physical properties: b.p. at 740 mm.=240° C.; d^{25}_4=1.128; n^{25}_D=1.4228; molecular weight in phenanthrene = 116.

	Calc.	Found
Percentage C	51.7	51.5
" H	6.96	7.10

[1] Doctoral thesis number 745, submitted June 5, 1944.

The compound is colorless, insoluble in water, very faint in odor, and a solvent for cellulose esters, all characteristics which suggest its possible utilization as a plasticizer in the plastics and elastomer industry.

Yields of butadiene from both the ether and the inner carbonate of 2,3-butylene glycol were poor. Yields of 13 per cent of theoretical were realized from the monomethyl ether of the glycol, and yields of only 1 per cent were realized from the carbonate. Evidently the inner carbonate, which is a heterocyclic compound, is not as readily pyrolyzed as are the ordinary dialkyl carbonates, which Ritchie (1935) pyrolyzed to unsaturated hydrocarbons.

Methyl, vinyl ketone

The production of methyl vinyl ketone, a material which produces excellent copolymers with butadiene, was investigated. The following chemicals were tried as starting materials for the preparation of methyl vinyl ketone: (1) 2,3-butylene glycol, (2) methylvinylcarbinol, and (3) acetylmethylcarbinol. A method of analysis for methyl vinyl ketone in mixtures with the various impurities present was developed. The analysis employed the polarographic method and was based on the fact that methyl vinyl ketone is reducible at the dropping mercury electrode, whereas the impurities present are not reducible and do not affect the reduction of the methyl vinyl ketone.

The 2,3-butylene glycol proved to be an unsatisfactory source of methyl vinyl ketone. Yields of 5 per cent of theoretical were realized by the simultaneous catalytic dehydration and oxidation of the glycol using a catalyst composed of NaH_2PO_4 on asbestos.

Methyl vinyl ketone yields of 65 per cent of theoretical were produced by the catalytic oxidation of methylvinylcarbinol, using a catalyst composed of zinc oxide (100 g.) and cupric oxide (30 g.). The individual components of the catalyst were inactive but when combined formed a catalyst active at a temperature of 250°C., which is quite low for the catalytic oxidation of unsaturated alcohols. Cuprous oxide was as effective as cupric oxide but was not easily maintained in that particular state of oxidation. Catalytic dehydrogenation of methylvinylcarbinol produced mixtures of methyl vinyl ketone and methyl ethyl ketone, the former being produced in yields of about 30 per cent of theoretical.

The highest yields of methyl vinyl ketone from acetylmethylcarbinol were 13 per cent of theoretical. In general, alcohols having a negative group adjacent to the hydroxyl group dehydrate to unsaturated compounds with difficulty, and acethylmethylcarbinol is such an alcohol. Furthermore, the acetylmethylcarbinol has a great tendency to reduce components of the catalyst, and components such as WO_3, W_2O_5, and $NaHSO_4$ were found to be unsuitable because of this reason.

Methyl acrylate

Methyl acrylate has received considerable attention for production of plastics, and Ziegler (1938) stated that it could be used to form copoly-

mers with butadiene. In recent years, a process for the production of methyl acrylate from lactic acid, a fermentation product, has been developed. This process involves esterification of methyl lactate with acetic acid and pyrolysis of the α-acetoxypropionate at a temperature of about 500° C., a procedure devised by Burns, Jones, and Ritchie (1935) and studied more recently by the staff of the Eastern Regional Research Laboratory. The possible esterification with phosgene instead of acetic acid and pyrolysis of the carbonate instead of the acetate was investigated. Results were encouraging in that a solid polymer could be produced at a relatively low temperature but were discouraging in that the monomer could not be isolated.

Diacetyl

Diacetyl has not been utilized to any great extent in elastomers but is capable of forming polymers with dialdehydes. The production of diacetyl by catalytic oxidation of 2,3-butylene glycol was investigated, and an analytical method employing the polarographic technique was developed. Yields of diacetyl corresponding to 40 per cent of theoretical were attained using a catalyst composed of zinc oxide (100 g.) and cupric oxide (30 g.). Air was used to effect the oxidation and the amounts of air required corresponded to only 1 mol of oxygen per 4 mols of hydroxyl groups.

The utilization of diacetyl for production of polymers was also investigated, and it was demonstrated that diacetyl would condense with either formaldehyde or glyoxal at a pH of 8.0. With formaldehyde a viscous liquid, very probably a mixture of polyhydric diketo compounds was formed, and with glyoxal a solid polymer, suitable for a varnish material, was produced.

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THE FERMENTATIVE PRODUCTION OF GLYCEROL[1]

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INTRODUCTION

The formation of glycerol during the course of an alcoholic fermentation has been known since the time of Pasteur (1858), but it was not until the period of the first World War that attempts were made to alter the course of the fermentation so as to produce greater quantities of glycerol. Neuberg and his co-workers (1917, 1919) in their investigations on the mechanism of the alcoholic fermentation of sugars by yeast found that acetaldehyde was one of the intermediates in this process. If the acetaldehyde was prevented from being reduced to ethyl alcohol by the addition of sodium sulfite to the medium, glycerol appeared in the medium in quantities equivalent to the amount of aldehyde bound by the sulfite. On the basis of this work the following equation was developed,

(1) $C_6H_{12}O_6 \rightarrow C_3H_8O_3 + CH_3CHO + CO_2$.

As can be seen from this equation, 1 mol of acetaldehyde and 1 mol of glycerol are formed for each mol of hexose fermented.

Connstein and Lüdecke (1921) in Germany and Cocking and Lilly (1922) in England developed processes for the production of glycerol by fermentation based on equation (1). The German process was actually in operation during the last war. The major difficulty with the process appeared not to be in the fermentation itself, but in the recovery of the glycerol from the fermented material.

Neuberg had also shown that in alkaline solution the acetaldehyde underwent a dismutation to produce ethyl alcohol and acetic acid. The overall fermentation reaction was represented as proceeding according to the following equation,

(2) $2\ C_6H_{12}O_6 + H_2O \rightarrow C_3H_8O_3 + C_2H_5OH + CH_3COOH + 2\ CO_2$.

In the United States Eoff (1918) obtained a patent covering the production of glycerol by fermenting a sugar solution in the presence of sodium carbonate. In neither the sulfite nor the alkaline methods for the production of glycerol by fermentation did the yields of glycerol reach the theoretical yields demanded by the equations developed by Neuberg. This failure was attributed to the fact that the normal alcoholic fermentation proceeded simultaneously with the modified fermentations.

There have been numerous other patents and reports in the literature concerning the production of glycerol by fermentation, but most of the improvements have only been modifications of the processes described previously. Hickey (1941) suggested the use of the relatively insoluble magnesium sulfite in place of the more soluble sodium sulfite. The use of the slightly soluble sulfite would simplify the recovery of the glycerol

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since most of the sulfite could be readily removed after the fermentation was completed.

With few exceptions, no reports were found in the literature on the use of starchy materials, converted to fermentable sugars, as substrates for the fermentative production of glycerol. Most of the previous work has involved the use of purified sugars, such as sucrose or dextrose, or molasses. In this thesis a study was made of the possibility of using starchy materials as sources of fermentable sugars for the production of glycerol by the sulfite processes.

EXPERIMENTAL

Since the sulfite process for the production of glycerol by fermentation was the only one studied in this work, the reaction between acetaldehyde and the sodium or magnesium bisulfite during fermentation furnished an indirect but rapid method for the determination of the amount of glycerol formed during the fermentation. The method is a modification of the one developed by Tomoda (1929). By iodine titration the amount of free sulfite is determined in a slightly acid solution, and the titration is continued in alkaline solution to determine the amount of sulfite that was bound by the acetaldehyde formed during the fermentation. The acetaldehyde-bisulphite complex is almost completely dissociated in alkaline solution. The amount of sulfite determined in the second part of the titration is equivalent to the amount of acetaldehyde formed which in turn is equivalent to the amount of glycerol present according to equation (1).

Both magnesium sulfite and sodium sulfite were used in this work, and for fermentable substrates enzyme-converted starchy materials, acid-hydrolyzed starchy materials, and purified sugars were employed. It was found that enzyme-converted starchy materials were not satisfactory for the fermentative production of glycerol. Maltose, as the pure sugar, was found to be fermented very slowly by yeast in the presence of sulfites, and since maltose is the chief sugar produced by the action of diastase on starch, the poor fermentations of the enzyme-converted starchy materials were readily understandable. In order to ferment maltose with yeast in the presence of sulfite, the medium must have a pH of 6.9 to 7.1, and suitable nutrients must be present. A 5 per cent maltose solution containing 4 per cent sodium sulfite required 15 days to reach completion, the yield being about 20 per cent glycerol on the initial sugar. A dextrose fermentation under similar conditions was completed in 3 days with a comparable yield of glycerol.

The acid-hydrolysis of corn starch or dry-milled corn products furnished solutions which fermented satisfactorily in the presence of sulfites. By using magnesium sulfite the highest yields of glycerol fell in the range from 22 to 24 per cent glycerol on dextrose, agreeing with the yields obtained by Hickey (1941). With sodium sulfite, yields up to 30 per cent glycerol were obtained, but for yields above 25 per cent high concentrations of sulfite and excessively large yeast inocula were necessary.

The effect of the concentration of sulfite, the concentration of sugar (dextrose), and of the amounts of yeast used for inocula on the yields of glycerol were studied, and all of these factors were found to be interrelated. In several fermentations the pH changes during the course of the fermentation were observed by means of a Cameron automatic pH recorder. The strain of yeast used seemed to have little effect on the yields of glycerol when dextrose or sucrose was the sugar being fermented, but a strain of *Saccharomyces ellipsoideus* apeared to be the best for the fermentation of maltose. It was also found that the temperature and the surface-volume ratio of the fermenting medium affected the yields of glycerol somewhat. An increase of 7° C. in the incubation temperature increased the yield of glycerol by 0.6 per cent. Surface-volume ratios greater than 0.2, a condition usually found only in very small scale fermentations, apparently decreased the yields of glycerol. In general it may be said that the factors which influence the rate of an enzymatic reaction will influence the amounts of glycerol formed by fermentation.

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CUPROUS OXIDE AS A CATALYST: THE EFFECT OF VARYING THE PROPORTIONS OF PROMOTER AND STABILIZER[1]

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The hydrogenation of furfural to furfuryl alcohol in the liquid phase, using promoted cuprous oxide catalysts, was first reported by Menzel (1) in 1936. Of the promoters investigated, calcium oxide, mechanically added to the Cu_2O, was found to be the best. Stewart (2) extended the use of the cuprous oxide-calcium catalyst to the hydrogenation of acetophenone. He found the mechanical addition of vanadium tetroxide to the catalyst mixture was necessary in order to stabilize the cuprous oxide against reduction to inactive copper. The V_2O_4 also promoted the Cu_2O-CaO catalyst for the hydrogenation of furfural. The adsorptive capacities of promoted cuprous oxide catalysts and their catalytic activity have been investigated by Stanerson (3). The following catalysts, arranged according to decreasing ability, were active in adsorbing hydrogen at 57° C. or higher: Cu_2O-BaO, Cu_2O-V_2O_4-CaO, and Cu_2O-CaO. Cuprous oxide promoted with SrO or MgO adsorbed little or no hydrogen. The Cu_2O-V_2O_4-CaO mixture was considered the best catalyst from the standpoint of rate of reaction, temperature at the start of reaction, adsorptive capacity, and stability against reduction. By determining the optimum proportions of these three ingredients it was hoped to extend the scope of the catalyst. The influence of alkaline earth oxides on the Cu_2O-V_2O_4 mixture was investigated by hydrogenating acetophenone and furfural.

EXPERIMENTAL

A Parr hydrogenation apparatus, Model B3B, of 0.5 liter capacity was used. The weighed quantities of Cu_2O and the promoters, mechanically ground, were inserted into the bomb along with 0.5 mole of hydrogen acceptor. Hydrogen was admitted to an initial pressure of 1,000 lbs./ sq. in. The heater and oscillator were started, and time, temperature, and pressure readings were recorded every 5 minutes. When the temperature of the bomb reached 200° C. the heater was disconnected, and readings continued until the temperature fell to 150°C. at which time the bomb was immediately cooled to room temperature. The pressure readings were converted to absolute pressure at 0°C. in order to have comparable basis for hydrogen consumed. Graphs were constructed for each run plotting decrease in pressure against time, which gave the desired comparison of the rate of hydrogenation. The rate of reaction, arbitrarily defined as the time required for the consumption of 0.45 mole of hydrogen,

[1] Doctoral thesis number 751, submitted June 7, 1944.

the temperature at the start of the reaction, and the total moles of hydrogen adsorbed gave additional comparisons of catalytic activity.

The Cu_2O, V_2O_4, and Cr_2O_3 were prepared according to the directions of Stewart. The oxides of Mg, Ca, Sr, and Ba were used as promoters. Adkins' (4) copper-chromium oxide catalyst was used for comparisons.

RESULTS

A. Calibration of the bomb was found to be necessary for obtaining the actual moles of hydrogen consumed by the hydrogen acceptor. Acetone was hydrogenated quantitatively to isopropyl alcohol. It was found that 0.055 mole of hydrogen was retained by the contents of the bomb over and above that used for complete hydrogenation.

B. Analysis of a carefully dried sample of the Cu_2O catalyst gave approximately 4 per cent water, 94 per cent Cu_2O, and 2 per cent copper as metal. The V_2O_4 was found to be hydrated.

C. Increasing the amount of V_2O_4 in the catalyst mixture of Cu_2O-V_2O_4-CaO lowered the temperature at which reaction started and increased the rate of reaction when furfural was used, but lowered the rate of hydrogenation of acetophenone. Amounts of V_2O_4 greater than half the amount of Cu_2O produced little improvement and are not necessary. The V_2O_4 stabilized the Cu_2O-CaO catalyst in the hydrogenation of acetophenone.

D. Increasing the amount of CaO in the catalyst mixture of Cu_2O-V_2O_4-CaO had a more pronounced effect than increasing the amount of V_2O_4. Increase in the amount of CaO lowered the temperature at the start of the reaction, increased the rate of reaction, and produced a higher yield of hydrogenated product in a shorter time interval when either furfural or acetophenone was used. Furfural containing water reacted more slowly than dry furfural when Cu_2O-V_2O_4-CaO was used, but the activity of the catalyst was decreased and not destroyed.

E. The catalyst Cu_2O-Cr_2O_3-CaO was slightly more active than Cu_2O-V_2O_4-CaO in the hydrogenation of furfural but less active in the hydrogenation of acetophenone.

F. Copper-chromium oxide catalyst of Adkins was less active than Cu_2O-V_2O_4-CaO in the hydrogenation of furfural but more active in the hydrogenation of acetone and acetophenone. Adkins' catalyst was more active in the hydrogenolysis of acetophenone to ethylbenzene than Cu_2O-V_2O_4-CaO.

G. Of the alkaline earth oxides calcium oxide was the best promoter for Cu_2O-V_2O_4 in the hydrogenation of furfural to furfuryl alcohol and acetophenone to methylphenylcarbinol. Magnesium oxide promoted Cu_2O-V_2O_4 in the hydrogenation of furfural but produced more highly hydrogenated products. The MgO and BaO were inactive as promoters for Cu_2O-V_2O_4 in the hydrogenation of acetophenone. Strontium oxide had some promoter action in both hydrogenations. Barium oxide promoted Cu_2O-V_2O_4 rather remarkably in the hydrogenation of furfural but did not produce complete hydrogenation.

CONCLUSIONS

1. The comparison of catalytic activity of catalysts containing Cu_2O-V_2O_4-CaO required careful drying of each constituent and the control of as many variables as possible.

2. A cuprous oxide catalyst prepared by Menzel's method contained approximately 94 per cent Cu_2O, 4 per cent H_2O, and 2 per cent copper as metal.

3. Calibration of the Parr hydrogenation apparatus was necessary in order to determine the moles of hydrogen actually consumed by the hydrogen acceptor.

4. Increasing the proportion of V_2O_4 in the catalyst Cu_2O-V_2O_4-CaO up to a weight ration of Cu_2O to V_2O_4 of 10 to 5 (optimum) lowered the temperature at which reaction started and increased the rate of hydrogenation of furfural. The addition of V_2O_4 to Cu_2O-CaO decreased the rate of hydrogenation of acetophenone but stabilized the catalyst against reduction.

5. Increasing the proportion of CaO in the catalyst Cu_2O-V_2O_4-CaO was found to be very advantageous in producing an active catalyst. The lowering of the temperature at the start of the reaction and the increase in the rate of the reaction were appreciable.

6. The Cu_2O-V_2O_4-CaO was more active for hydrogenating the aldehyde group than the ketone group in the compounds investigated. This catalyst was not as active as Adkins' copper-chromium oxide catalyst for hydrogenolysis of organic compounds.

7. Calcium oxide was the best alkaline earth oxide used as a promoter for Cu_2O-V_2O_4 in the hydrogenation of furfural and acetophenone.

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THE SYNTHESIS OF o-HYDROXYALDEHYDES[1]

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Studies on the synthesis of o-hydroxyaldehydes were undertaken in an effort to find a more satisfactory method for the laboratory and commercial preparation of this class of compounds. The o-hydroxaldehydes are of interest, being yellow in color, and volatile with steam, properties probably derived from their "hydrogen bridge" structure, and in forming "chelate ring" compounds with the metals. The aldehyde group in this class of compounds is remarkably stable toward alkali and quite resistant to oxidation. It does, however, condense readily with primary amines to give Schiff's bases which in turn form chelate compounds with several metals. Salicylaldoxime, the oxime derivative of the most common of the o-hydroxyaldehydes, salicylaldehyde, has found rather extensive use as an analytical reagent.

Nearly all of the o-hydroxyaldehydes reported in the literature have been prepared by means of the Reimer-Tiemann reaction. This reaction consists in the action of chloroform on a phenol in a strongly alkaline solution. The method involves several time-consuming operations and gives very low yields. It is particularly unsatisfactory for the preparation of o-hydroxyaldehydes from *ortho*-substituted phenols, the yields being in general less than 10 per cent. The method is useless when o-halogen- or o-nitrophenols are employed. Although many modifications of the Reimer-Tiemann reaction have been suggested previous to this work, no systematic study has been reported of the effect of varying the amounts of reagents used over a considerable range in order to determine the optimum conditions for the reaction. Such a study of the Reimer-Tiemann reaction has now been made using o-ethoxyphenol. Two series of reactions were run in which the chloroform concentration was varied from 0.8 to 8.0 moles per mole of phenol. Another series of reactions was conducted in which the only variable was the alkali concentration. As a result of this study the optimum proportions of reagents were found to be 4 moles of chloroform, 9 moles of alkali, and 80–85 moles of water to 1 mole of the phenol. The best yield obtained, however, was only 10 per cent.

A series of reactions was carried out in an autoclave employing pressures of 150–250 p.s.i.g. It was found that the yields by the Reimer-Tiemann method may be increased only a few per cent by carrying out the reaction at a pressure of 150–175 p.s.i.g.

Another method of synthesizing o-hydroxyaldehydes, the allyl rearrangement method, has been used to a very limited extent as a laboratory method. This method involves four principal steps, namely, prepara-

[1] Doctoral thesis number 733, submitted November 15, 1943.

tion of the allyl ether of the phenol, rearrangement of the allyl ether to the *o*-allylphenol, isomerization to the *o*-propenylphenol, and finally, oxidation of the propenyl group to an aldehyde. Although this method is somewhat involved the yields obtained over the first three steps in the synthesis are excellent. The oxidation step, however, has been found difficult. The method was considered too long and involved to be a convenient laboratory method.

A new and apparently general method for the preparation of *o*-hydroxyaldehydes was described recently by Duff. This reaction consists in bringing together hexamethylenetetramine and a phenol in the presence of anhydrous glycerol and glyceroboric acid at a temperature of 160°. Initial attempts to use this method in the preparation of several other *o*-hydroxyaldehydes were unsuccessful. Control of temperature in the reaction was not extremely important. The critical step was the manner of adding the phenol and hexamethylenetetramine to the hot glyceroboric acid mixture. Best results were obtained if the two reactants were added simultaneously. Otherwise the phenol, when added alone, polymerized rapidly with the glycerol, and the hexamethylenetetramine if added alone decomposed rapidly at the high temperature. This slight modification in the original procedure resulted in a marked increase in the yields obtained. As a laboratory synthesis the method of Duff was found to be far superior to the Reimer-Tiemann reaction, the time required being only a fraction of that necessary in the Reimer-Tiemann method. The method was found applicable to *ortho*-substituted phenols including halogenated phenols. The yields obtained by the Duff reaction ranged from 10 to 40 per cent, a marked improvement over the Reimer-Tiemann method.

Adopting the modified Duff procedure, the following *o*-hydroxyaldehydes have been prepared: 2-hydroxy-3,6-dimethylbenzaldehyde, 2-hydroxy-4,6-dimethylbenzaldehyde, 2-hydroxy-3,5-dimethylbenzaldehyde, 2-hydroxy-5-methylbenzaldehyde, 2-hydroxy-3-bromo-5-*tert*-butylbenzaldehyde, 2-hydroxy-3-*iso*-propyl-6-methylbenzaldehyde, 2-hydroxy-5-phenylbenzaldehyde, 2-hydroxy-3-bromobenzaldehyde, 2-hydroxy-3-chlorobenzaldehyde, 2-hydroxy-5-ethylbenzaldehyde, 2-hydroxy-3-*n*-butoxybenzaldehyde, 2-hydroxy-3-*tert*-amylbenzaldehyde, 2-hydroxy-3-methyl-5-*tert*-amylbenzaldehyde, 2-hydroxy-3-chloro-5-*tert*-butylbenzaldehyde, 2-hydroxy-3-*iso*-propyl-5-chloro-6-methylbenzaldehyde, and 2-hydroxy-3,5-dibromobenzaldehyde. The procedure was also applied to several phenols from which two different o-hydroxyaldehydes might be formed. It was not definitely established whether the products obtained in these instances were mixtures of the two possible isomers or single compounds. These phenols were as follows: 3,4-dimethylphenol, 2-methyl-4-chlorophenol, 3-methyl-4-*tert*-butylphenol, and 2-hydroxy-4-*tert*-butylphenol.

With the exception of a few aldehydes originally reported by Duff the above aldehydes have not been previously prepared by this method. Several have not been heretofore reported in the literature. The compounds were carefully purified, appropriate physical constants determined, and derivatives prepared.

The Schiff's bases formed by the condensation of these *o*-hydroxyaldehydes and ethylenediamine are yellow crystalline compounds with sharp melting points and may therefore serve as derivatives for the identification of these aldehydes. These derivatives are more easily prepared and recrystallized than are the other common derivatives such as semicarbazones, oximes, and phenylhydrazones.

Aldehydes were not obtained by the application of the Duff reaction to 2-nitrophenol, 2,4-dinitrophenol, and 2-hydroxypyridine.

OPERATING CONDITIONS FOR OPTIMUM BEHAVIOR OF A CONTINUOUS COUNTERCURRENT, COUNTERGRAVITY EXTRACTION PLANT[1]

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Soybeans have increased at a rapid rate in both agricultural and industrial importance during the past few years. While many products are made from soybeans the principal products are the oil and the meal. The oil is used largely for edible purposes and to a lesser extent in paints, printing ink, linoleum, and soap. The meal is a high protein stock feed.

Soybean oil may be pressed out or dissolved out with a suitable solvent. The solvent process removes 95 per cent or more of the approximately 20 per cent oil present in the beans, while the pressure process seldom removes over 75 per cent. Several solvent extraction systems, differing in details, are in commercial use. These systems use commercial hexane as a solvent. Hexane is a good solvent for soybean oil and is low in first cost but, being flammable, presents a definite explosion hazard.

Various nonexplosive solvents have been considered for the extraction of vegetable oils such as soybean. Of these trichloroethylene appears to be the most attractive. It is an excellent solvent for soybean oil. It is nonflammable, low in corrosive action on steel, and easily removed from the oil and meal.

A review of the literature indicates that trichloroethylene vapors have a toxic action, although some of the symptoms ascribed to it may be due to the presence of impurities in the commercial product. By the use of suitable precautions the health hazards may be reduced to a negligible amount.

Analytical methods for the determination of oil in the beans, oil in the meal, moisture in the beans, moisture in the meal, oil in the miscella, trichloroethylene in the oil, and trichloroethylene in the meal have been selected and adapted to the control of the process.

Experimental studies have been carried out in semicommercial equipment described in a thesis by Kircher (1). The extractor consisted of two lengths of steel pipe joined at an angle of about 60 degrees with the longer pipe at an angle of about 15 degrees with the horizontal. The flaked beans moved down the longer pipe countercurrent to the movement of the solvent which entered part way up the shorter leg. The extracted meal was freed of solvent in a jacketed three-section drier. Screw conveyors were used in both the extractor and the drier to move the flakes continuously forward. Two tubular heat exchangers (the evaporator and stripper) were used to remove the solvent from the oil. The solvent vapors from the driers and the stripper were condensed in a surface condenser.

[1] Doctoral thesis number 727, submitted August 3, 1943.

After preliminary runs to check the general operation of the equipment, a series of runs was made to determine desirable changes in equipment and operating procedure. Significant problems and their solutions will be summarized.

Flaking the beans was the first step in the extraction process. Beans cracked into several pieces in a roll crusher were flaked between a pair of smooth rolls. Since some finely powdered material which caused difficulties in operation was always produced during the flaking, studies were made of means of reducing it. Best results were secured from beans tempered by adjusting the moisture and temperature with steam before flaking. This tempering rendered the beans more plastic, thus reducing the fine material to a minimum. A maximum thickness of 15 thousandths of an inch was found desirable for maximum extraction in minimum time.

Even with careful flaking sufficient fine material was carried from the extractor into the evaporator and stripping still to cause difficulty with foaming and bumping and to interfere with the proper stripping of the solvent from the oil. In an effort to remove the fines, a settling tank was installed between the vaporizer and the still. While enough of the fines settled out in this tank to increase the length of time the equipment could be successfully operated, the use of a settling tank, which would require a considerable investment in extra solvent and equipment, was not considered to be a solution of the problem. A new arrangement of the vaporizer and the still was made. The kettles at the bottom of the vaporizer and still were removed. The former was placed directly over the latter with a 1-foot-long section of pipe with a side outlet between. The bottom end of the still dipped into a special bucket kept full of oil to act as a seal. Under this new arrangement the miscella filmed down over the tubes in the vaporizer and still, and the oil and the bulk of the fines passed out the bottom into the sealing bucket.

Two miscella filters were eventually installed. Each of these was a bag filter of cloth suspended in a casing of 12-inch pipe with a removable flanged cover. Only one filter was used at a time allowing plenty of time for emptying the other. These filters operated very satisfactorily.

The original screw conveyors in the driers and steamer were of the ribbon type using a $\frac{3}{8}$-inch rod instead of the conventional flat ribbon. The wet meal, entering the top drier from the extractor by gravity, accumulated in the inside of the ribbon and rotated with it instead of moving forward. To remedy this about 5 feet of the ribbon screw were removed and replaced with solid flight screw. Similarly about 2 feet of solid flight conveyor was installed at the entry ends of the second drier and the steamer. Later the solid flight in the second drier was reduced to 1 foot following the formation of a plug in this drier, believed to have been caused by too great a length of solid flight.

In the original arrangement the solvent vapors from each of the two driers and the steamers were carried by separate vapor lines to the condenser above the driers. Considerable difficulty was experienced by the clogging of vapor lines and fouling of the condenser with meal dust. It

was found, after several changes, that the most satisfactory arrangement was to use one vapor line of large size connecting the outlet end of the upper drier to the condenser. Since the solvent vapors are heavier than air the condenser was placed below the level of the driers. To prevent the loss of solvent vapor from the steamer, the meal was originally discharged through a homemade barrel valve, but this stuck on several occasions when meal dust coated the moving bearing surface. Attempts to replace the valve by a conical hopper with a bottom discharge gate were unsuccessful. The problem was solved by procuring a commercially made barrel valve which functioned excellently.

The flaked beans were originally fed into the extractor at a point below the liquid level, but experience showed that more satisfactory results could be obtained by feeding in just above the liquid. A rotameter for measuring the solvent going into the extractor was found to be desirable.

Operation of the equipment under good conditions indicated that the oil content of the meal could be reduced to 1 per cent and the solvent loss to less than 1 per cent of the beans processed. The steam required was about 1 pound per pound of beans, and the water required was about 1.5 gallons per pound of beans.

It is believed that commercial equipment designed on the basis of the data secured could be operated successfully for processing soybeans.

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CHANGES IN PALATABILITY, MICROSCOPIC APPEARANCE, AND ELECTRICAL RESISTANCE IN BEEF DURING THE ONSET AND PASSING OF RIGOR AND DURING SUBSEQUENT STORAGE [1]

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A study was made of the effect of storage at 34–36°C. on a yearling steer, and of the differences between the principal muscles of the round of this animal. The storage times used were 0, 1, 2, 4, 9, and 18 days. The muscles utilized were the semitendinosus, semimembranosus, biceps femoris, the three vasti, adductor, and gastrocnemius of the round, and the psoas major of the loin. A balanced incomplete block design was employed in assigning the storage times to the various cuts and in analyzing the data.

The muscles were separated, cut into roasts, wrapped in pliofilm and stored in the Animal Husbandry meat cooler. After the appropriate period of storage, they were taken out for testing. The raw roasts were inspected for any changes due to storage and for differences between the muscles. The electrical conductivity and the pH were determined for the raw meat. The cuts were then roasted in 150°C. ovens. The maximum internal temperature of the roasts averaged 66°C., with a range of 65 to 67°C. The appropriate weights and measurements were taken so that the total, dripping, and evaporation losses, change in volume, and cooking time per pound could be calculated.

After the internal temperature started to drop, the roasts were sampled for judging and objective tests. The palatability factors of tenderness, juiciness, aroma, and flavor of lean and fat were scored by four judges. The objective tests included the force required to shear a cylinder of meat 1 inch in diameter, and the amount of fluid expressible by 250 pounds pressure in 5 minutes.

Samples of both raw and cooked meat were made into microscopic slides for histological study. Cross and longitudinal sections were made of gelatin-imbedded tissues, using the freezing microtome, and of paraffin-imbedded tissues, using the rotary microtome. The frozen sections were stained to differentiate between the muscle fibers and the fat, while the paraffin sections were stained to show the muscle fibers and the two types of connective tissue—collagenous and elastic.

The slides were inspected for changes produced by cooking muscle not yet in rigor, by normal rigor, and by aging in cold storage. The frozen cross sections were used for measurement of the fiber diameters and counts for the number of fibers per bundle in the various muscles.

Supplementary studies were made on a pair of psoas major cuts to

[1] Doctoral thesis number 724, submitted July 9, 1943.

check on the effect of massage before the muscle went into rigor, and on small strips of neck muscle to check the effect of exposure to 70°C. for various times in producing heat rigor.

Inspection of the raw roasts showed that the amount of leakage of juice was greatest during the second to fourth days of storage. By the eighteenth day, the roasts were sticky on the surface and rather "high" in aroma, indicating bacterial activity. This was most noticeable in the vasti, which also attained the highest pH.

The grain and amount of external and internal fat varied from muscle to muscle. The psoas major was the finest in texture, the gastrocnemius and vasti were next, the semitendinosus and adductor were medium grained, and the semimembranosus and biceps femoris were coarse. Only the semitendinosus and the biceps femoris had a continuous covering of external fat on one side. The gastrocnemius had some, and the other muscles very little external fat.

The electrical resistance of the raw meat dropped sharply with storage, while the pH first decreased, then increased as the storage time lengthened.

Only one of the fresh roasts, the psoas major, went into rigor before being cooked. It had passed through the stage of maximum stiffness by the end of the cooking period. The other fresh roasts were not in rigor before cooking, but were in rigor when cooked. All the stored roasts had passed out of rigor before being cooked.

The total cooking losses did not vary significantly with increased storage or between the muscles. The amount of dripping loss increased sharply from the ninth to the eighteenth day of storage, but this was offset by a corresponding decrease in the loss by evaporation. The muscles with large amounts of external fat had somewhat higher dripping losses than those with little or no outside fat covering.

The volume of the roasts decreased with cooking. The cooking time was not changed by storage, but the smaller roasts required more minutes per pound than the larger ones.

The judging scores for tenderness, juiciness, flavor of lean, and aroma increased generally from 0 to 9 days of storage, then either remained about stationary or decreased somewhat. The decrease in scores for flavor of lean and aroma after 18 days of storage was due to the development of "gaminess" in the meat which was considered definitely undesirable by some of the judges. When judgments of the flavor of the fat were possible, the scores indicated little change up to 9 days of storage, with a decided decrease in desirability after 18 days of storage. This decrease was due to the development of rancidity.

The force required to shear the meat decreased with storage, the drop being especially marked from the fresh roasts to those stored 1 day. The press fluid dropped during the early part of the storage period, then increased decidedly during the last 9 days.

The variation in tenderness between the different muscles as indicated by judging scores and by shear showed that the psoas major was

the most tender muscle used, the gastrocnemius and adductor being next. The four large muscles of the round, the semitendinosus, semimembranosus, vasti, and biceps femoris, did not differ significantly from each other in tenderness.

The diameter of the muscle fibers decreased with cooking but not with storage. The variation between the muscles was quite large, the biceps femoris, vasti, and gastrocnemius having the largest fibers and the semitendinosus and psoas major the smallest. The number of fibers per bundle was too variable to show any consistent change with storage or between the different muscles.

Microscopically, the freshly-killed meat had straight to slightly wavy fibers, which were poorly differentiated. Storage for 1 day led to the appearance of rigor nodes and crinkled or kinked fibers. The nodes persisted throughout the storage time, but the crinkles and kinks tended to disappear, being replaced by sharp breaks in the fibers. The stretched areas of the fibers immediately adjacent to the rigor nodes frequently disintegrated, leaving a granular residue. Cooking brought on the appearance of rigor in the fresh meat, and increased the number of broken fibers in the meat stored 9 and 18 days. The rigor nodes produced in the fresh meat by cooking were not as dense as those found in the stored meat.

The change in collagen caused by cooking could be observed in the slides as a loss of ability to take the acid fuchsin stain and a change from the normal fibrous state to a somewhat granular residue.

Massaging the freshly-killed muscle before cooking apparently speeded up the onset of rigor, but caused little other change except in tenderness, the score of the massaged roast being 3.25 points lower, and the shearing force 18 pounds higher, than that of the control.

Exposure of small strips of fresh muscle to 70°C. gave the typical picture of extreme heat rigor, the fibers showing alternate bands of maximum contraction and rarefaction. Two seconds exposure to this heat affected only the outer fibers, but within 10 seconds the fibers were changed throughout the sample of tissue.

DERIVATIVES OF PHENOTHIAZINE AS CHEMOTHERAPEUTIC AGENTS [1]

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Derivatives of phenothiazine have been selected for study of their chemotherapeutic properties because of the low cost of phenothiazine, its low toxicity to higher animals, and its high toxicity to lower animals, and the fact that in several cases phenothiazine has already been shown to possess chemotherapeutic value. Chief interest in this investigation has been centered on the antimalarial properties of phenothiazine derivatives.

The structural formula of phenothiazine with the numbering system in present use is given in Fig. 1. A survey of the known substitution re-

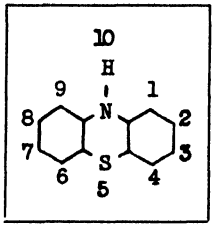


Fig. 1

actions of phenothiazine and 10-substituted derivatives shows that substitution occurs generally in the 3-position. The only exception to this rule previously known is the metalation of 10-ethylphenothiazine with *n*-butyllithium.

A survey of the biological applications of phenothiazine indicates that it has found satisfactory use as an insecticide, as a urinary antiseptic, and as an anthelmintic agent in human and veterinary medicine. A few derivatives of phenothiazine have shown activity as antimalarial agents.

In this investigtion, phenothiazine was shown to undergo metalation by *n*-butyllithium in the 1-position in a yield of 52 per cent. The reactions involved in the proof of the position of the entering lithium atom are shown in Fig. 2. The identity of the ketones VIII and XIII indicates that the position of the entering metal atom was *ortho* to the nitrogen atom.

The metalation of 10-phenylphenothiazine and 10-ethylphenothiazine by *n*-butyllithium was shown to involve either the 2- or the 4-position in the phenothiazine nucleus. The metalation reaction and the reactions used in proving the positions of the entering lithium atoms are shown in Figs. 3 and 4. In the case of the metalation of 10-phenylphenothiazine, the possibility of metalation in the *meta* position on the 10-phenyl group was excluded by the synthesis of this acid and a comparison of its properties with those of the metalation acid. Of the two possible positions for the

[1] Doctoral thesis number 735, submitted December 14, 1943.

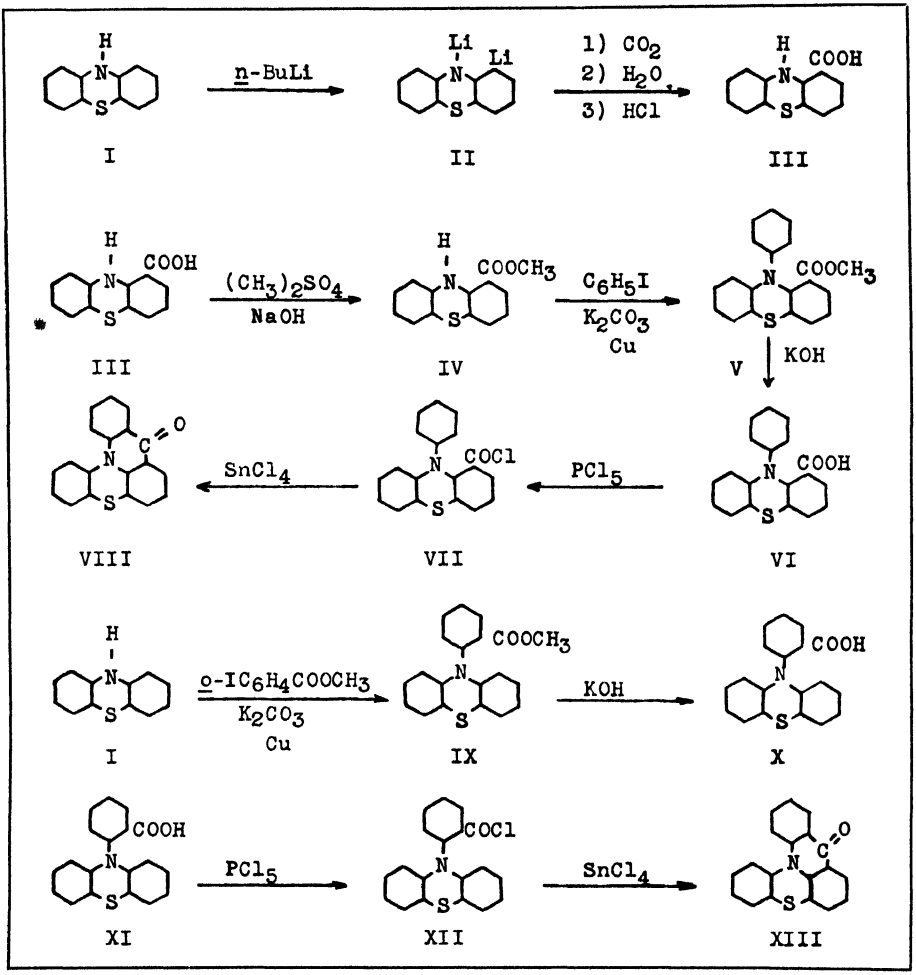

n-BuLi
1) CO2
2) H2O
3) HCl
(CH3)2SO4
NaOH
C6H5I
K2CO3
Cu
KOH
PCl5
SnCl4
o-IC6H4COOCH3
K2CO3
Cu
KOH
PCl5
SnCl4
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII

Fig. 2

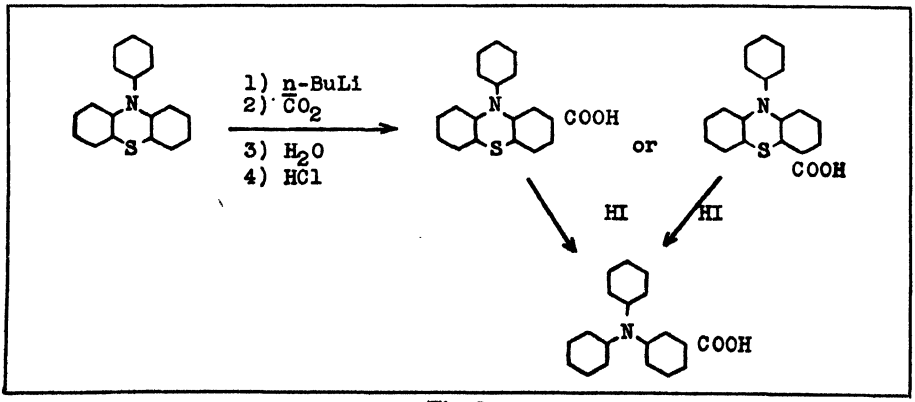

1) n-BuLi
2) CO2
3) H2O
4) HCl
or
HI
HI

Fig. 3

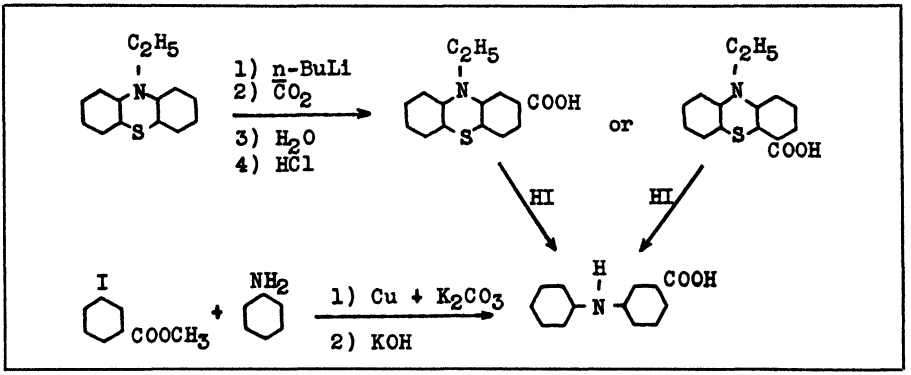


Fig. 4

metalation of 10-phenylphenothiazine and 10-ethylphenothiazine, the 4-position, or the one *ortho* to the sulfur atom, is considered the more likely because of the tendency in metalation reactions with *n*-butyllithium of the lithium atom to enter a heterocyclic nucleus in a position *ortho* to the hetero atom.

The derivatives of phenothiazine which were prepared for pharmacological testing may be divided into the following three groups: (1) derivatives of 10-phenylphenothiazine which contains a γ-diethylaminopropylamino group on the 10-phenyl radical, (2) derivatives of phenothiazine containing a dialkylaminoalkyl group in the 10-position, and (3) miscellaneous derivatives, some of which were prepared as intermediates in the preceding syntheses.

The derivatives of 10-phenylphenothiazine containing the diethylaminopropylamino group were synthesized by the series of reactions shown in Fig. 5, in which the preparation of 10-(2′-γ-diethylaminopropylamino-4′-methoxy)phenylphenothiazine serves as an example. The compounds synthesized by these reactions were 10-(2′-γ-diethylaminopropylamino) phenylphenothiazine (b.p. 215–230°/0.5 mm.), 10-(2′-γ-diethylaminopropylamino-4′-methyl)phenylphenothiazine (the product distilled at a bath temperature of 270° at a pressure of less than 0.5 mm.), 10-(4′-γ-diethylaminopropylamino)phenylphenothiazine (the product distilled at a bath temperature of 350° at a pressure of less than 0.5 mm.), 10-(2′-γ-diethylaminopropylamino-4′-methoxy)phenylphenothiazine (b.p. 220–235°/0.5 mm.), and 10-(2′-γ-diethylaminopropylamino-4′-chloro)phenylphenothiazine (b.p. 270–280°/2 mm.). All of these compounds were yellow, fluorescent, viscous oils. None showed definite antimalarial activity.

The series of 10-dialkylaminoalkylphenothiazine derivatives was prepared by the reactions shown in Fig. 6 in which the preparation of 10-γ-diethylaminopropylphenothiazine serves as an illustration. The compounds prepared by these reactions were 10-β-diethylaminoethylphenothiazine (b.p. 161–165°/0.5 mm.), 10-β-di-*n*-propylaminoethylphenothiazine (b.p. 225–230°/1 mm.), 10-β-morpholinoethylphenothiazine (m.p. 74.5–75.5), 10-β-(6′-methoxy-8′-quinolyl)aminoethylphenothiazine (m.p. 118.5–

119.5°), 10-γ-diethylaminopropylphenothiazine (b.p. 170–182°/0.5 mm.), 10-γ-di-*n*-propylaminopropylphenothiazine (b.p. 257–262°/2 mm.), 10-γ-diallylaminopropylphenothiazine (b.p. 245–260°/1 mm.), 10-γ-piperidylpropylphenothiazine (b.p. 255–265°/1–2 mm.), 3-methoxy-10-γ-di-*n*-pro-

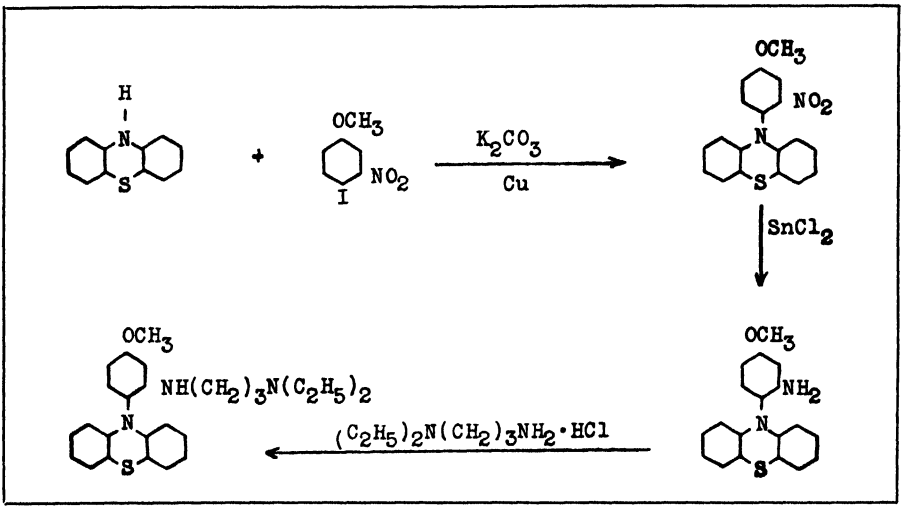


Fig. 5

pylaminopropylphenothiazine (b.p. 250–265°/2 mm.), and 3-methoxy,10-γ-diethylaminopropylphenothiazine (b.p. 220–225°/0.5 mm.). This series of compounds was also without antimalarial activity.

The miscellaneous derivatives of phenothiazine which were not

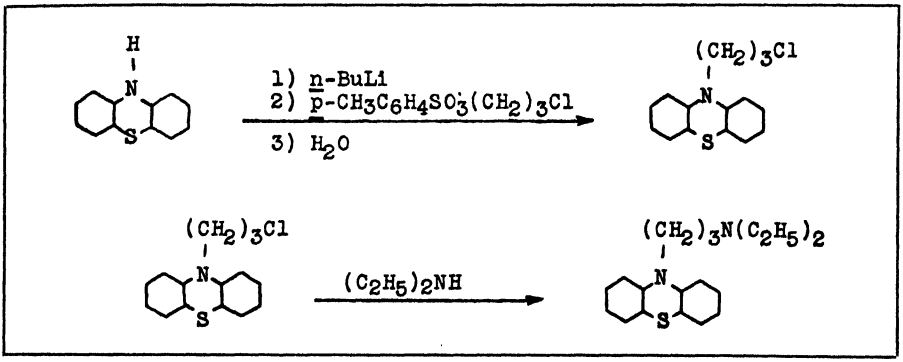


Fig. 6

included as intermediates in the above syntheses are 3-methylphenothiazine (m.p. 160–162°), 1-carboxyphenothiazine (m.p. 264–264.5°), 10-(4′-carboxy)phenylphenothiazine (m.p. 221–221.5°), 3-nitro-10-phenylphenothiazine-5-oxide (m.p. 223.5–224.5°), 10-β-chloroethylphenothiazine (m.p. 96–97°), 10-allylphenothiazine (b.p. 187–195°/1 mm.), 10-decyl-

phenothiazine (b.p. 183–185°/0.5 mm.), and 10-octadecylphenothiazine (m.p. 53°).

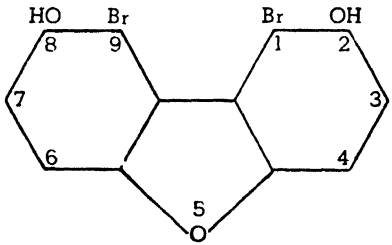
In spite of a low toxicity to higher animals and a high toxicity to lower animals found in the parent compound, derivatives of phenothiazine prepared in this investigation showed no definite activity when tested against malarial parasites in the avian blood stream.

1,9-DERIVATIVES OF DIBENZOFURAN[1]

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Bromination of 2,8-dihydroxydibenzofuran by Swislowsky[2] gave the supposed 1,9(?)-dibromo- derivative (I). Many of the following transformations were made in attempts to prove or disprove this structure.



I

2,8-Dihydroxydibenzofuran was dibrominated by the method of Swislowsky[2] and, wtihout purification, the product was acetylated giving 1,9(?)-dibromo-2,8-diacetoxydibenzofuran. 1,9(?)-Dibromo-2,8-dimethoxydibenzofuran was nitrated in acetic acid at 85°, giving *1,9(?)-dibromo-2,8-dimethoxy-3(?)-nitrodibenzofuran,* m.p. 243–244°. The same product was obtained by the use of acetyl nitrate in acetic anhydride. 1,9(?)-Dibromo-2,8-dimethoxydibenzofuran in acetic acid was refluxed with two equivalents of nitric acid for 1½ hours. A small yield of *1,9(?)-dibromo-2,8-dimethoxy-3,7(?)-dinitrodibenzofuran,* m.p. 222–223°, was obtained. Heating 1,9(?)-dibromo-2,8-dimethoxydibenzofuran on a steam bath with a mixture of equal volumes of acetic acid and fuming nitric acid gave *1,9(?)-dibromo-2,8-dimethoxy-3,4,7(?)-trinitrodibenzofuran,* m.p. 212–214°.

2,8-Dimethoxydibenzofuran was heated with fuming nitric acid, giving 2,8-dimethoxy-1,3,7,9(?)-tetranitrodibenzofuran, m.p. 246–247°, which had been made previously by a different method[3]. The latter was reduced by Raney nickel and hydrogen at 4 atmospheres, giving *2,8-dimethoxy-1,3,7,9(?)-tetra-aminodibenzofuran,* m.p. 181–182°. Acetylation of the tetra-amino-compound gave *1,9(?)-diamino-2,8-dimethoxy-3,7(?)-diacetaminodibenzofuran,* m.p. 295–296°.

1,9(?)-Dibromo-2,8-dimethoxy-3(?)-nitrodibenzofuran was demethylated by hydrobromic acid in glacial acetic acid, giving *1,9(?)-dibromo-2,8-dihydroxy-3(?)-nitrodibenzofuran,* m.p. 267–268°. The latter was nitrated in glacial acetic acid giving *1,9(?)-dibromo-2,8-dihydroxy-3,7(?)-*

[1] Doctoral thesis number 736, submitted December 14, 1943.
[2] Swislowsky, Doctoral Dissertation, Iowa State College. (1939).
[3] Willis and Yeoman, unpublished studies.

dinitrodibenzofuran, m.p. 204°. The same product was obtained by direct dinitration of 1,9(?)-dibromo-2,8-dihydroxydibenzofuran.

2,8-Dimethoxydibenzofuran was mononitrated in acetic acid giving *1(?)-nitro-2,8-dimethoxydibenzofuran*, m.p. 158–159°, and *3(?)-nitro-2,8-dimethoxydibenzofuran*, m.p. 172–174° in the ratio of approximately four parts of the latter to one of the former.

2,8-Dihydroxy-3,7(?)-dibromodibenzofuran-1,9(?)-dicarboxylic acid[4] was treated with diazomethane, giving *2,8-dimethoxy-3,7(?)-dibromo-1,9(?)-dicarbomethoxydibenzofuran*, m.p. 230–231°, which was saponified by sodium methoxide and water in methanol to give *2,8-dimethoxy-3,7(?)-dibromodibenzofuran-1,9(?)-dicarboxylic acid*, m.p. 322–324°. Decarboxylation of the latter by heat was not successful. 2,8-Dihydroxy-3,7(?)-dibromodibenzofuran-1,9(?)-dicarboxylic acid in dioxan was esterified by methanol and anhydrous hydrogen chloride giving *2,8-dihydroxy-3,7(?)-dibromo-1,9(?)-dicarbomethoxydibenzofuran*, m.p. 268–269°. Decarboxylation of 2,8-dihydroxy-3,7(?)-dibromodibenzofuran-1,9(–)-dicarboxylic acid using copper and quinoline resulted in removal of the bromine atoms also, giving 2,8-dihydroxydibenzofuran.

2,8-Dimethoxydibenzofuran-1,9(?)-dicarboxylic acid[2] was treated with fuming nitric acid. The product was a mixture, melting at 247–249°, after two crystallizations from dilute acetone. Esterification of this crude product by methanol and dry hydrogen chloride gave *2,8-dimethoxy-3,4,6,7(?)-tetranitro-1,9(?)-dicarbomethoxydibenzofuran*, m.p. 199.5–200°.

A caustic fusion of 1,9(?)-dibromo-2,8-dimethoxydibenzofuran followed by acetylation gave the acetoxy- compound, melting at 174.5–175.5°, which was converted to a methoxy- derivative, melting at 100–101°. The products were not identified.

1,9(?)-Dibromo-2,8-dimethoxydibenzofuran was treated with one equivalent of butyllithium and then with water giving *3-bromo-2,8-dimethoxydibenzofuran*, m.p. 117.5–118°, whose structure has recently been proved.[5]. When the reaction was repeated using dimethyl sulfate in place of water, *1-methyl-2,8-dimethoxy-7(?)-bromodibenzofuran*, m.p. 144–145°, was obtained. The same product (mixed m.p.) was obtained previously.[5]. These reactions indicated that the supposed 1,9-derivative is, probably, 1,7-dibromo-2,8-dimethoxydibenzofuran.

2,8-Dihydroxydibenzofuran was converted to *2,8-diallyloxydibenzofuran*, m.p. 70–71°, and to *2,8-dibenzofuryloxyacetic acid*, m.p. 271–273°. One attempt to rearrange the allyloxy- compound was not successful.

2-Amino-3-bromodibenzofuran was brominated giving *1(?),3-dibromo-2-aminodibenzofuran*, m.p. 181.5–182°. The same product was obtained by direct bromination of 2-aminodibenzofuran. Diazotization of the amino group and replacement by the hydroxyl group gave *1(?),3-dibromo-2-hydroxydibenzofuran*, m.p. 112–113°. Bromination of 2,8-

[4] Yeoman, unpublished studies.
[5] Hogg, unpublished studies.

diaminodibenzofuran gave polybromo derivatives which could not be purified.

3-Nitrodibenzofuran gave, when incompletely reduced by hydrogen and Raney nickel, 3,3′-azodibenzofuran, m.p. 268–270°. Borsche and Schacke[6] reported a melting point of 282°.

3-Aminodibenzofuran was refluxed with o-chloronitrobenzene, potassium carbonate, copper bronze, and nitrobenzene giving *o-nitrophenyl-3-dibenzofurylamine,* m.p. 139.5–140°. 3-Aminodibenzofuran was treated with chloroacetic acid and sodium hydroxide to give *3-dibenzofurylglycine,* m.p. 139–142°, which was esterified to *3-dibenzofurylglycine methyl ester,* m.p. 123–124°.

1,2,4-Trimethoxybenzene was metalated by butyllithium and carbonated to give 2,3,6-trimethoxybenzoic acid, m.p. 149–150°. This acid was previously synthesized by Smith and LaForge[7] and was found to melt at 145–146°. A mixture of these two products melted at 146–149°. The acid was esterified to *methyl 2,3,6-trimethoxybenzoate,* m.p. 57–57.5°, and to *ethyl 2,3,6-trimethoxybenzoate,* m.p. 42.5–43°.

Iodination of the metalation product of 1,2,4-trimethoxybenzene gave *2,3,6-trimethoxyiodobenzene,* m.p. 108–108.5°, which was coupled by the Ullmann reaction to give *2,2′,3,3′,6,6′-hexamethoxybiphenyl,* m.p. 125–125.5°. The latter compound was nitrated by acetyl nitrate in acetic anhydride to give *2,2′,3,3′,6,6′-hexamethoxy-5,5′-dinitrobiphenyl,* m.p. 151–151.5°.

2,3,6-Trimethoxyiodobenzene was nitrated to give *2,3,6-trimethoxy-5-nitroiodobenzene,* m.p. 119.5–120°, which, on shaking with hydrogen and palladium-calcium carbonate catalyst, gave the known 2,4,5-trimethoxynitrobenzene[8] (mixed m.p.). Coupling 2,3,6-trimethoxy-5-nitroiodobenzene using copper powder gave 2,2′,3,3′,6,6′-hexamethoxy-5,5′-dinitrobiphenyl (mixed m.p.).

Ring-closure attempts on the hexamethoxybiphenyl derivatives were unsuccessful.

[6] Borsche and Schacke, Ber., 56:2498 (1923).
[7] Smith and LaForge, Jour. Am. Chem. Soc., 53:3072 (1931).
[8] Schuler and Thoms, Arch. Pharm., 245, 267, 276 (1907). [*Chem. Zentr. II,* 806 (1907)].

FREE RADICALS IN THE DECOMPOSITION OF ORGANOMETALLIC COMPOUNDS[1]

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Organometallic compounds undergo thermal, electrolytic, and photochemical decompositions. Free radicals exist as intermediate products in these decompositions. Coupling, disproportionation, and formation of the RH compound by the abstraction of hydrogen from the solvent, are the principal reactions of the free radicals. Most coupling reactions, using metal salts, are considered to operate through free radicals.

Metal-metal interconversions are characteristic of many organometallic derivatives. The reaction is represented by the following general equation:

$$RM^1 + R'M^2 \rightarrow R'M^1 + RM^2$$

Both silver bromide and silver iodide reacted with dimethylmagnesium to give good yields of pure ethane. The reaction was very rapid.

Methyllithium with gold tribromide produced a 76.8 per cent yield of ethane and an 18.4 per cent yield of methane. Since other experiments have shown that trimethylgold normally decomposes to give pure ethane, the methane undoubtedly resulted because of a catalytic action which altered the mode of breakdown of the trimethylgold.

Zirconium tetrachloride reacted with both methylmagnesium iodide and dimethylmagnesium to give pure methane.

With methylmagnesium chloride, tantalum pentachloride formed a good yield of pure methane.

Dimethylmagnesium reacted with chromium trichloride to give pure methane (62.0 per cent yield).

A catalytic amount of gold tribromide (5 mole per cent) effected a coupling of methyllithium with methyl iodide to give ethane. Some methane was produced in the process, but the formation of that compound was probably due to a side-reaction.

Ferric chloride, ferrous chloride, and nickelous chloride catalyzed the reaction of bromobenzene with phenylmagnesium bromide to give yields of biphenyl varying between 26 and 45 per cent based on the total amount of phenyl groups available.

Refluxing dimethylgold bromide in ether for 48 hours produced pure ethane, metallic gold, methylgold dibromide, and gold tribromide. A disproportionation of dimethylgold bromide undoubtedly occurred, giving trimethylgold, methylgold dibromide, and gold tribromide. Being thermally unstable, trimethylgold then decomposed to give pure ethane and metallic gold.

Diphenylcadmium, refluxed in benzene for 82 hours, thermally de-

[1] Doctoral thesis number 723, submitted July 8, 1943.

composed to give only a small yield (3.3 per cent) of biphenyl. However, cadmium chloride reacted with benzylmagnesium chloride to give a 77 per cent yield of the coupling product, bibenzyl.

The preparation of diphenylantimony chloride by reacting antimony trichloride with phenylmercury bromide was attempted. The experiment was unsuccessful and a quantitative recovery of the phenylmercury bromide was realized.

The following satisfactory preparation of diphenylantimony chloride was developed. Triphenylantimony was dearylated by reaction with hydrogen peroxide[2] to give diphenylstibonic acid. This stibonic acid was dissolved in hot, moderately concentrated hydrochloric acid to give diphenylantimony trichloride, the insoluble impurities were removed by filtration, and the diphenylantimony trichloride reduced to diphenylantimony chloride with stannous chloride. The diphenylantimony chloride was purified by crystallization from acetic acid. This process is more dependable and more simple than other preparations of diphenylantimony chloride.

The preparation of phenyl-*p*-tolylstibonic acid by the reaction of phenylantimony oxide with benzenediazonium chloride, and the subsequent alkaline decomposition, was attempted. The desired product could not be isolated.

Tri-*p*-chlorophenylantimony was prepared in a 71.5 per cent yield by the reaction of *p*-chlorophenylmagnesium bromide with antimony trichloride. The compound was crystallized from a chloroform-methanol solution, and melted at 101.0–101.5°.

Tri-*p*-chlorophenylantimony dichloride was prepared in almost a quantitative yield by slowly bubbling chlorine gas through a solution of tri-*p*-chlorophenylantimony in ice-cold chloroform. The product was crystallized from a chloroform-methanol solution. The compound melted at 189.5–190.5°.

Tri-(*p*-dimethylaminophenyl)antimony was prepared in a 35 per cent yield by the reaction of *p*-dimethylaminophenyllithium with antimony trichloride. The crystallization was carried out in an ethanol-chloroform solution. The maximum melting point of the compound was 239–241°.

Both triphenylantimony dichloride and tri-*p*-tolylantimony dichloride were quantitatively reduced by hydrazine hydrate[3] in 95 per cent ethanol to give triphenylantimony and tri-*p*-tolylantimony, respectively.

Hydrazine hydrate apparently did not react with diphenylantimony chloride and *p*-tolylantimony dichloride to give the R_3Sb compounds.

Metal-metal interconversions were carried out on symmetrical and unsymmetrical triarylantimony compounds in accordance wih the following equation:

$$R_3Sb + n\text{-}C_4H_9Li \rightarrow R_2SbC_4H_9\text{-}n + RLi$$

[2] Goddard, "Organometallic compounds," which constitutes Part III of Vol. XI of Friend, "A text-book of inorganic chemistry," Charles Griffin Co., London (1936), p. 239.

[3] Hydrazine hydrate had previously been used to convert arylbismuth halides to R_3Bi compounds by Gilman and Yablunky. Jour. Am. Chem. Soc., 62:665 (1940).

The amount of RLi compound was determined by carbonation to give the corresponding carboxylic acid. The solution of 0.005 mole of the antimony compound and 0.005 mole of *n*-butyllithium in 60 cc. of anhydrous ether was stirred for 10 minutes before carbonation. These conditions were comparable to those used in the experiments with the corresponding organobismuth compounds[4].

Triphenylantimony gave benzoic acid in yields of 8.3, 11.4, and 9.8 per cent. Tri-*p*-tolylantimony produced 5.9 and 6.5 per cent yields of *p*-toluic acid. Tri-*p*-chlorophenylantimony gave *p*-chlorobenzoic acid in yields of 38.0 and 36.9 per cent. Diphenyl-α-naphthylantimony gave a 15.5 per cent yield of α-naphthoic acid; only a trace of benzoic acid was formed. Diphenylmesitylantimony produced a 10.2 per cent yield of benzoic acid; no 2,4,6-trimethylbenzoic acid was produced. Diphenyl-*p*-chlorophenylantimony gave a 20.2 per cent yield of *p*-chlorobenzoic acid, and a 3.9 per cent yield of benzoic acid.

The arrangement of the series of radicals in the decreasing amount of cleavage is as follows: *p*-chlorophenyl, α-naphthyl, phenyl, *p*-tolyl, and mesityl. The series is in entire accord with that based on the cleavage of the unsymmetrical triarylbismuth compounds.[4b]

On the basis of cleavage from unsymmetrical mercurials by hydrogen chloride, the above radicals possess the following order of decreasing lability: mesityl, α-naphthyl, *p*-toyl, phenyl, and *p*-chlorophenyl[5]. It is very apparent that a cleavage series of this type depends upon both the metal derivative being cleaved and the cleaving agent.

The appearance of a transitory green color in the reaction of the liquid ammonia solution of diphenylantimony iodide with metallic sodium may be taken as evidence for the formation of diphenylantimony.

$$(C_6H_5)_2SbI + Na \rightarrow (C_6H_5)_2Sb + NaI$$

The same general observations were made with the corresponding bismuth compound[6].

[4](a) Gilman, Yablunky, and Svigoon, *ibid.*, 61:1170 (1939); (b) Gilman and Yale, *Chem. Rev.*, 30:281 (1942).

[5] Kharasch, Legault, and Sprowls, Jour. Org. Chem., 3:409 (1938); Kharasch and Flenner, Jour. Am. Chem. Soc., 54:674 (1932).

[6] Gilman and Yablunky, Jour. Am. Chem. Soc., 63:212 (1941).

SYNTHETIC DIBENZOFURANS STRUCTURALLY RELATED TO KNOWN ANALGESICS[1]

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Emphasis in recent work on dibenzofuran derivatives has been placed upon the synthesis of 1- and 1,9- substituted compounds which might serve as intermediates in the preparation of products containing a bridge between the 1- and 9-positions. Swislowsky[2] brominated 2,8-dimethoxydibenzofuran to obtain two dibromo isomers which he designated tentatively as 1,9(?)-dibromo-2,8-dimethoxydibenzofuran and 2,8-dimethoxy-3,7(?)-dibromodibenzofuran. The work of Willis[3] has provided strong evidence in support of the structure of the latter compound. A considerable portion of the present studies has been devoted to a completion of the proof of structure of 2,8-dimethoxy-3,7(?)-dibromodibenzofuran and to a further investigation of the structure of 1,9(?)-dibromo-2,8-dimethoxydibenzofuran.

Nitration of 2,8-dihydroxydibenzofuran gave 1,3,7,9(?)-tetranitro-2,8-dihydroxydibenzofuran, m.p. 246–247°, which upon methylation yielded 1,3,7,9(?)-tetranitro-2,8-dimethoxydibenzofuran, m.p. 245–246°. A mixture of the dihydroxy and dimethoxy compounds melted at 218–219°,

A modification of the procedure of Swislowsky[2] for the preparation of 1,9(?)-dibromo-2,8-dimethoxydibenzofuran from 2,8-dihydroxydibenzofuran was introduced. The crude 1,9(?)-dibromo-2,8-dihydroxydibenzofuran obtained from bromination of 2,8-dihydroxydibenzofuran was acetylated to give pure 1,9(?)-dibromo-2,8-diacetoxydibenzofuran, m.p. 173–174°, which was methylated directly to yield 1,9(?)-dibromo-2,8-dimethoxydibenzofuran, m.p. 195–196°. The overall yield for the conversion of 2,8-dihydroxydibenzofuran to 1,9(?)-dibromo-2,8-dimethoxydibenzofuran was 44.3 per cent.

When attempts at bromination of 2,8-dimethoxydibenzofuran-1,9(?)-dicarboxylic acid were unsuccessful, the acid was cleaved to 2,8-dihydroxydibenzofuran-1,9(?)-dicarboxylic acid, m.p. 313–314°, which was dibrominated to yield 2,8-dihydroxy-3,7(?)-dibromodibenzofuran-1,9(?)-dicarboxylic acid, m.p. 318–319°. Decarboxylation of the latter acid was attempted by treatment with a suspension of powdered copper in quinoline. No pure product was obtained, although Thirtle[4] obtained from the same reaction a product which may have been crude 2,8-dihydroxydibenzofuran.

Preparation of 1,9(?)-diamino-2,8-dimethoxydibenzofuran was attempted by treatment of 1,9(?)-dibromo-2,8-dimethoxydibenzofuran with cuprous bromide and ammonium hydroxide in a steel bomb. The amine

[1] Doctoral thesis number 742, submitted March 16, 1944.
[2] Swislowsky, Doctoral Dissertation No. 540, Iowa State College (1939).
[3] Willis, Doctoral Dissertation No. 712, Iowa State College (1943).
[4] Thirtle, Doctoral Dissertation No. 736, Iowa State College (1943).

obtained proved too unstable for purification. Only starting material was recovered from the treatment of 1,9(?)-dibromo-2,8-dimethoxydibenzofuran with sodamide in liquid ammonia.

Conversion of 1,9(?)-dibromo-2,8-dimethoxydibenzofuran to 2,8-dimethoxydibenzofuran-1,9(?)-dialdehyde, m.p. 237–238°, was accomplished in 62 per cent yield by halogen-metal interconversion with *n*-butyllithium followed by treatment with N-methylformanilide. The aldehyde was characterized by preparation of the dioxime, m.p. 243–244°. Oxidation of the aldehyde yielded 2,8-dimethoxydibenzofuran-1,9(?)-dicarboxylic acid, whose dimethyl ester proved identical by mixed melting point with an authentic sample of 1,9(?)-dicarbomethoxy-2,8-dimethoxydibenzofuran. Attempts were made to prepare cyclic compounds from 2,8-dimethoxydibenzofuran-1,9(?)-dialdehyde by condensations with hydrazine and with o-phenylenediamine. Only products of polymeric nature resulted.

The crude product obtained from dibromination of 2-hydroxydibenzofuran[5] was acetylated to yield 1,x-dibromo-2-acetoxydibenzofuran, m.p. 154–155°. Monobromination of 1-bromo-2-hydroxydibenzofuran[5] followed by acetylation yielded a dibromo compound identical with the above 1,x-dibromo-2-acetoxydibenzofuran, thus establishing the location of the bromine atom in the 1-position. Hydrolysis of 1,x-dibromo-2-acetoxydibenzofuran yielded 1,x-dibromo-2-hydroxydibenzofuran, m.p. 181–182°. The 1-bromo-2-hydroxydibenzofuran used in the above monobromination was prepared by a modification of the method of Van Ess[5]. The crude product obtained from the monobromination of 2-hydroxydibenzofuran was acetylated to give 1-bromo-2-acetoxydibenzofuran, m.p. 135–136°, which was then hydrolyzed to pure 1-bromo-2-hydroxydibenzofuran. Anomalous results were obtained in the attempted cleavage of 2-methoxy-3-bromodibenzofuran to yield 2-hydroxy-3-bromodibenzofuran. The alkali-soluble product obtained melted at 168–170° as compared with a melting point of 143–144° reported by Van Ess for an authentic sample of 2-hydroxy-3-bromodibenzofuran prepared from 2-amino-3-bromodibenzofuran. Methylation of the anomalous cleavage product yielded a white, alkali-insoluble compound, m.p. 154–155°, which was obviously not 2-methoxy-3-bromodibenzofuran.

The crude 1(?)-bromo-2,8-dihydroxydibenzofuran obtained from entrainment bromination of 2,8-dihydroxydibenzofuran acetylated to yield 1(?)-bromo-2,8-diacetoxydibenzofuran, m.p. 142–144°. Methylation of the latter compound gave 1(?)-bromo-2,8-dimethoxydibenzofuran, m.p. 102.5–103.5°.

Treatment of 4-dibenzofuryllithium with *n*-butoxymethylpiperidine gave 4-dibenzofuryl-N-piperidinomethane, b.p. 175–180° (0.5 mm.), which gave a picrate of m.p. 177–178°.

From a diazo coupling of *m*-trifluoromethylaniline with 2-hydroxydibenzofuran was obtained 1-(*m*-trifluoromethylphenylazo)-2-hydroxydibenzofuran, m.p. 173–174°, and a similar coupling reaction with 2,8-dihydroxydibenzofuran yielded 1-(*m*-trifluoromethylphenylazo)-2,8-di-

[5] Gilman and P. R. Van Ess, Jour. Am. Chem. Soc., 61:1365 (1939).

hydroxydibenzofuran, m.p. 256–257°. No pure product could be isolated from the reaction of two equivalents of *m*-trifluoromethylbenzenediazonium chloride with 2,8-dihydroxydibenzofuran.

Treatment of *m*-trifluoromethylphenylmagnesium bromide[6] with N-methylformanilide gave *m*-trifluoromethylbenzaldehyde, b.p. 64–66° (10 mm.), n^{20}_D 1.4660, d^{20} 1.300. From the aldehyde was prepared the oxime, b.p. 102–104° (12 mm.), n^{20}_D 1.5128, d^{20} 1.305, and the 2,4-dinitrophenylhydrazone, m.p. 259–260°. Treatment of 4-aminodibenzofuran[2] with *m*-trifluoromethylbenzaldehyde yielded 4-(*m*-trifluoromethylbenzalamino)-dibenzofuran, m.p. 81–83°. From *m*-trifluoromethylbenzaldehyde and *m*-trifluoromethylaniline was prepared 3-(*m*-trifluoromethylbenzalamino)-benzotrifluoride, m.p. 50–51°.

Attempts at purification of the dibenzofuran-4-aldehyde obtained from reaction of 4-dibenzofuryllithium with N-methylformanilide were unsuccessful. From the crude aldehyde was prepared dibenzofuran-4-aldehyde 2,4-dinitrophenylhydrazone, m.p. 301–302°.

Condensation of acetonylacetone with *p*-aminoacetanilide gave N-(*p*-acetaminophenyl)-2,5-dimethylpyrrole, m.p. 207–208°. Hydrolysis of the acetamino compound gave an amine which was too unstable for purification. No pure product was isolated from the attempted diazo coupling of the crude amine with 2-hydroxydibenzofuran.

Treatment of 5-iodotoluhydroquinone dimethyl ether[7] with cuprous cyanide gave 2,5-dimethoxy-*p*-tolunitrile, m.p. 130–131°. Hydrolysis of of the nitrile gave 2,5-dimethoxy-*p*-toluic acid, m.p. 125–126°. The same acid was prepared from 5-iodotoluhydroquinone dimethyl ether by halogen-metal interconversion with *n*-butyllithium followed by carbonation. Oxidation of 2,5-dimethoxy-*p*-toluic acid gave 2,5-dimethoxyterephthalic acid, m.p. 265–265.5°, which was converted to the diethyl ester, m.p. 101–102°. The melting points obtained for 2,5-dimethoxyterephthalic acid and its diethyl ester agree with those reported by Nef[8]. It has thus been demonstrated that iodination of toluhydroquinone dimethyl ether involves the 5-position, and the ring-closure synthesis of 2,8-dihydroxy-3,7-dimethyldibenzofuran by Willis[3] has been validated.

The 5-nitrotoluhydroquinone dimethyl ether reported by Erdtman was reduced to the corresponding amine which proved too unstable for satisfactory purification. Acetylation of the crude amine yielded 5-acetaminotoluhydroquinone dimethyl ether, m.p. 160–162°. The diazonium salt obtained from diazotization of 5-aminotolyhydroquinone dimethyl ether was converted to an iodo compound identical with 5-iodotoluhydroquinone dimethyl ether. Nitration of toluhydroquinone dimethyl ether is therefore shown to involve the 5-position.

Nitration of 5-iodotoluhydroquinone dimethyl ether gave 5-nitrotoluhydroquinone dimethyl ether. Similarly, nitration of 2,5-dimethoxy-*p*-toluic acid resulted in an exchange of groups with the formation of 5-nitrotoluhydroquinone dimethyl ether.

[6] Simons and Ramler, Jour. Am. Chem. Soc., 65:389 (1943).
[7] Erdtman, Proc. Roy. Soc. (London), A143:191 (1933).
[8] Nef, Ann.: 258, 298 (1890).

DEVELOPMENT AND STRUCTURE OF *BROMUS INERMIS* LEYSS[1]

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Bromus inermis Leyss., the smooth brome grass, has attained prominence in recent years as a promising grassland and forage crop. The plant is known by a number of names: Hungarian brome, Austrian brome, Russian brome, awnless brome, and smooth brome. Its native habitat is variously said to be Eurasia, Russia, central Europe, and China. It was introduced into the United States in 1882 and has become a widely distributed and increasingly important crop. A knowledge of its development, morphology, and cytology will have direct application to problems relating to the development of improved strains.

REVIEW OF LITERATURE

Zherebina (75) (76) recognized two principal types of *Bromus inermis*, a steppe type and a meadow type. The meadow type was further subdivided into four subtypes: (1) tall, (2) bushy, (3) a prolific seed producer, (4) a type with short culms. The steppe type was found to be inferior to the meadow type in forage value and succulence, but superior in drought resistance. Physiological and morphological variations in seedlings as well as in members of the same clone were noted by Waldron (64). Differences were found in blade length and width, culm height, and crude protein content. Waldron (65) compared the coefficient of variation of smooth brome grass with that of other grasses and found a significant correlation only between height and weight.

Considerable variation in leafiness, height, habit of growth, rhizome development, heat and drought tolerance, disease resistance, and seed-producing qualities of smooth brome grass was noted by Frolick and Newell (27) who believed that much of the variability was environmental. Knobloch (40) found that six of eleven selected morphological characters in smooth brome grass varied to a greater degree than had been previously reported. The six characters were blade width, length of panicle, length of spikelet, lengths of both glumes and length of palea. The extensive literature dealing with variation in other grasses has been reviewed by Knob-

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[2] The writer expresses his appreciation to Dr. J. E. Sass for encouragement and advice throughout the course of the investigation. Thanks are due to Drs. G. J. Goodman and I. J. Johnson for assistance in the preparation of the manuscript and to Dr. C. P. Wilsie for the use of plants from the Iowa Agricultural Experiment Station plots.

loch (40). Beddows (10) reported that smooth grome grass is xenogamous but that pollination occurs between different spikelets of the same plant. After studying 121 strains, Keyser (39) concluded that many of them bred true. Some progenies, however, broke up into further strains.

Growth studies on brome grass have dealt principally with the root system. Weaver (69) found that the roots penetrated to a depth of about 4.7 feet. A depth of 5.5 feet for 2-year-old plants was recorded by Ten Eyck (61). A root length of 39 cm. after 12 months growth was measured by Gruber (30). Root lengths of as much as 287 cm. were found by Witte (73). Kannenberg (35) noted that after 2 years in sandy soil the roots of one plant covered 2.48 square meters.

Watkins (67) studied the effects of fertilizers, shade, competition and photoperiodism on brome grass. Fertilizers increased leaf production, the height and number of shoots and the dry weight of tops, but decreased the total number of rhizomes and the weight of the underground parts. Shade increased the number of elongated internodes, the height of the plants, and the nitrogen content, but lowered the carbohydrate content. A photoperiod of 18 hours increased the height and weight, the size of the rhizomes, and the percentage and absolute amounts of carbohydrates. Short-day plants had more shoots than long-day plants. When sown with alfalfa, the number of shoots and rhizomes and the dry weight increased.

Cytological work on *Bromus inermis* has consisted mainly of surveys of chromosome numbers in races or strains. Knobloch (40) has reviewed the literature on this phase. The species is known to have numerous forty-two-chromosome and fifty-six-chromosome races. One race having seventy-two chromosomes has been reported (49).

Morphological descriptions of Bromus are fragmentary. Sirrine (57) described the anatomy of the leaf in some detail. A cambiform meristematic zone in the nodes was described by Chrysler (21).

MATERIALS AND METHODS

The plants used in this study were obtained from the forage crops breeding nursery at the Iowa Agricultural Experiment Station, Ames, Iowa. These plants had been established from open-pollinated single plant selections and strains.

Strain 543-34 was used for the study of germination, seedling development, and for anatomical characteristics. This strain originated from a single-plant selection made at the Dominion Forage Crops Laboratory, Department of Agriculture, Saskatoon, Saskatchewan, Canada. After selective inbreeding, the third generation inbred progeny of the single plant was increased and distributed under the variety name Parkland.

For the anatomical study of the vegetative organs, most of the material was embedded in paraffin. Excessively hard or brittle tissues were embedded in celloidin. Microtome sections were stained most commonly in safranin-fast green. The hot water method of softening paraffin-embedded tissues made possible the sectioning of most tissues. Most of the drawings were made with the aid of a microprojector.

GERMINATION OF THE CARYOPSIS: DEVELOPMENT OF THE SEEDLING INTO AN ESTABLISHED PLANT

The order of emergence and gross features of the development of the vegetative organs are presented in the following descriptions. The developmental rates should be regarded as applicable only to the greenhouse conditions used in this study. Further studies should be made under controllable greenhouse conditions as well as under a range of field conditions.

First and second day after planting: The first observable evidence of germination is the swelling of the entire caryopsis. The radicle grows and pushes the coleorhiza against the pericarp. The coleorhiza then emerges near the base of the dorsal side of the caryopsis. The protruding coleorhiza bears white hairs, less than a millimeter in length. The primary root breaks out of the thin side of the coleorhiza rather than through the thicker tip and thus projects, for a time at least, at right angles to the median plane of the plumule.

Fifth day: Under the conditions prevailing in these plantings, the primary root was at least 18 mm. long and densely covered with root hairs except at the tip. The first adventitious root had made its appearance from the region of the scutellar node. The coleoptile was 10 mm. long and had developed chlorophyll. The first foliage leaf, 10 mm. long, had developed chlorophyll at the tip and white epidermal hairs on the inner and outer surfaces.

Fourteenth day: The primary root was as much as 80 mm. in length and had eight lateral roots. The scutellar adventitious roots were 30 mm. long and two or three roots had arisen at the coleoptile node. The first internode or "mesocotyl" appeared; the first foliage leaf was 85 mm. and the coleoptile about 20 mm. in length. The lemma, palea, and caryopsis were still adherent to the seedling.

Twenty-first day: The primary root bore numerous lateral roots, frequently in recognizable pairs. The scutellar root was about 37 mm. and the first internode 10 mm. in length. The first leaf had grown to 105 mm. and the second leaf to 72 mm. in length. The parenchymatous ligule on the first leaf was fully developed, 1 mm. long, and located approximately 20 mm. above the coleoptile node.

Twenty-eighth day: The primary root system comprised the largest part of the root system, having as many as thirteen large lateral roots. The largest adventitious root equaled the primary root in diameter, but was only about half as long. The adventitious roots from the coleoptile node emerged by breaking through the enveloping coleoptile.

Fifty-fourth day: The largest adventitious root in greenhouse plants was 150 mm. long; some seedlings had as many as three tillers and five leaves, and the coleoptile had shredded off.

One hundred eleventh day: As many as ten leaves were present, the lower six usually dead. The coleoptile node had become greatly swollen as a result of the formation and emergence of adventitious roots. A study of seedlings with three tillers showed that the best-developed tiller had a ruptured, two-veined prophyllum approximately 5 mm. long. This tiller

had two leaves, each with a distinct sheath 12 mm. long, and a blade 3 mm. long. Each leaf had a small ligule. Four other small, less-developed leaves were present. The second tiller also had a prophyllum 5 mm. in length. The first or basal leaf was approximately 18 mm. long, and the second leaf was at least 23 mm. long. Four other leaves were present, each having a blade and sheath. The third tiller, which was the smallest and youngest, had a prophyllum. The first leaf had a sheath 13 mm. long and a 2 mm. blade. The sheath of the second leaf was 23 mm. long, and the blade 5 mm. long. Roots had emerged from the bases of all three tillers.

One hundred eighty-ninth day: The primary roots were still present, but the remnants of the caryopsis were no longer attached to the plant. Between this date and the two hundred and twenty-first day, two short rhizomes developed, each less than 10 mm. long. The main culm had three tillers at its base, and each rhizome had three tillers at its apex. The plant was now well established, and showed evidence of spreading. No observations were made after this date.

GROSS MORPHOLOGY AND ANATOMY OF THE PLANT

PANICLE

The panicle in the strains studied varies in length from 80 to 200 mm. and consists of a central rachis and numerous secondary and tertiary branches. The panicle is wide-spreading, but occasionally it is unilateral (fig. 1). At maturity the panicle is somewhat contracted and purplish-brown, later changing to straw color. The secondary branches are arranged in fascicles, each fascicle constituting a half-whorl. The tertiary branches may occur singly or in half-whorls, and may branch again before ending in a spikelet. The rachis is usually glabrous, but the secondary and tertiary branches are covered with short, stiff, white, forward-pointing hairs.

Anatomically, the rachis is a hollow cylinder with two circles of vascular bundles, the outer circle having smaller bundles placed directly within the sclerenchymatous hypodermis (fig. 5). The larger bundles of the inner circle usually alternate with the smaller outer ones. Each bundle is composed of two to three protoxylem cells, two large flanking metaxylem vessels, xylem parenchyma, tracheids, phloem, and a thin bundle sheath.

The epidermis is heavily lignified, especially the outer cell walls. Masses of thin-walled chlorenchyma alternate with the thick-walled hypodermal groups around the periphery of the rachis, with stomates opening into the former. More chlorenchyma is found in the rachis than in the lower part of the culm. Bridges of thick-walled cells connect the lignified hypodermal groups under the chlorenchyma. The rest of the cortex is composed of thin-walled parenchyma.

In the secondary and subsidiary branches of the rachis the epidermal cells are also strongly thickened (fig. 6). There are two to three uninterrupted peripheral layers of hypodermal chlorenchyma and three to six layers of thick-walled sclerenchyma, usually containing four small, evenly-

spaced vascular bundles. The center of the branch is occupied by one to two larger bundles.

SPIKELET

The pedunculate spikelet usually ranges from 10–30 mm. in length and consists of a rachilla bearing two glumes, and two to ten florets. The upper one or two florets may be sterile, consisting of only a lemma, or a lemma and a palea, or a lemma and palea with reduced stamens and pistil.

The central axis of the spikelet is a disarticulating rachilla, each segment of which is about 3 mm. long and has a convex and a concave side, the latter facing toward the palea. Most of the rachilla is covered with stiff, white, upward-pointing hairs, and the distal end of each rachilla segment is oblique. At the base of the lemma, a mass of callus occurs, inseparable from and apparently fused to the rachilla.

GLUMES

The first or lower glume varies from 3 to 7 mm. in length and is smooth, acute, lanceolate, and has a prominent keel. Usually, one bundle is present, but occasionally three bundles occur. The chlorenchyma extends over the basal half of the glume, but distally it is confined to the bundle region. Stomates are present on both surfaces.

The internal anatomy of the first glume is similar to that of the palea and lemma (figs. 9–11). The outer or lower epidermal cells are heavily lignified on their rippled outer walls and on the radial walls, but less lignified on their inner tangential walls. Silicified cells occur scattered in the epidermis. The hypodermis may or may not be lignified. Below the hypodermis are one to three layers of thin-walled chlorenchyma. The inner or upper epidermal cells are rectangular and thin-walled. The glume margins are usually two cells in thickness. The bundle contains a few xylem elements, a large mass of phloem, and a bundle sheath which merges with the outer epidermis.

The second glume is broader and longer than the first glume, being from 4–9 mm. in length, and is smooth, acute, and lanceolate. Stomates are present on both surfaces, and the chlorophyll is distributed as in the first glume. The anatomy is similar to that of the first glume except that there are three vascular bundles (figs. 7–8). In the development of the spikelet, the glumes develop before the florets and enclose the rest of the young spikelet, while at maturity the glumes are much shorter than the rest of the spikelet.

LEMMA

The elliptical lemma ranges in length from 7–14 mm., and is thicker and more chlorophyllous than the palea. The lemma is convex on the outside, concave toward the pistil, its margin is translucent, and the surface is glabrous toward the apex and pubescent basally. The tip of the lemma is bifid and may have a chlorophyllous awn arising from the back. This awn is so near the apex that it appears to originate from between the

lemma tips. The awn may be 2 mm. in length. There are five to seven bundles, the center one and each alternate one being larger and hence more conspicuous than those in between (figs. 12–13). This pattern is similar to that of the blade. Chlorenchyma is abundant basally except in the margin, but distally is confined to the bundles. A thick callus occurs at the base of the lemma.

The outer epidermis is heavily lignified, especially on the rippled outer wall. Silicified cells and thickened hairs are also present. The hypodermis consists of one to two layers of cells, somewhat less lignified than the epidermis. Between the hypodermis and inner epidermis, one to three layers of thin-walled chlorenchyma cells extend throughout the interior of the lemma. The inner epidermis consists of rectangular, thin-walled cells without chloroplasts. Stomates occur on both surfaces. The margins of the lemma are composed of a mass of translucent sclerenchyma. The larger vascular bundles are like those of the palea, but the smaller ones have fewer xylem and phloem cells and a narrower bundle sheath.

PALEA

The palea is a bicarinate organ 5–14 mm. in length. It is concave on its dorsal side, and its "wings," each about 0.5 mm. wide, turn inward sharply, being concave on the ventral side (fig. 14). The keels, one on each side of the concavity, have forward-pointing hairs and may be prolonged upward into minute "teeth." The entire palea is minutely pubescent, more delicate in texture than the lemma, and is attached to the floret stalk, whereas the lemma is connected to the rachilla. Both lemma and palea adhere to the fruit in maturity.

The margins of the palea "wings" consist of a single layer of lignified cells which appear to be structurally similar to, and continuous with the hypodermal layer. The cell walls of the epidermis are thickest on the rippled external surface. Stomates were noted only in the outer epidermis. Below the hypodermis are one or two layers of thin-walled chlorenchyma, extending to the inner epidermis, which also consists of thin-walled cells.

Each of the structurally similar keels has a vascular bundle, and two to three layers of chlorenchyma (figs. 14, 15). The bundle sheath merges with the epidermis and does not have the endodermis-like layer characteristic of the leaf bundles. Between the keels, the palea is composed of the dorsal and ventral epidermis, with a nonchlorophyllous layer between. The amount of chlorenchyma is greater in the keels than in the margins or in the center.

LODICULES

The two lodicules are inserted on the flower stalk, lie at the basal portion of the inturned "wings" of the palea, and are shorter than the palea (fig. 16). During anthesis, they swell radially, pushing apart the lemma and palea and permitting the anthers to drop out, and the stigma to protrude. Vascularization is present in the lodicules, consisting mostly of xylem elements with annular thickenings.

STAMENS

The brome grass floret has three stamens, one of which is inserted between the two lodicules (fig. 16). The filaments are short at first, but before anthesis they increase several times in length by cell elongation. The filaments are composed mainly of parenchyma with much-elongated nuclei. Two or more annular vessels occur in each filament.

The two-lobed basifixed anther is about 4 mm. long and bifid at both ends. A small mass of tissue, the connectivum, lies between the lobes. The anther wall consists of four layers of cells, epidermis, endothecium, middle layer, and tapetum. The long axes of the epidermal cells are parallel to that of the anther. Stomates are present in the epidermis of the connectivum. The cells of the second or endothecial layer are elongated at right angles to those of the epidermal cells. Thickenings become evident in the endothecium, and this layer is responsible for the dehiscence of the anther. The middle layer has the long axis of its cells parallel with those of the epidermis. The tapetum is the innermost layer; its cells are at first uninucleate and later become binucleate by mitosis and the failure of cell wall formation.

The mature pollen grain is approximately spherical and has a granular exine and a thick intine. There is one germ pore, raised slightly above the surface. Fresh pollen was found to average 37.6 by 42.2μ, and when stained with aceto-carmine consistently shows two sperms and one tube nucleus. The sperms are 8.2 by 4.9μ, and the tube nucleus 13.2 by 8.2μ in diameter. Starch grains are present in the mature pollen grains.

PISTIL

The pistil is borne on a short stalk and consists of the ovary and two styles terminating in stigmatic papillae. The uniloculate ovary has a high, flattened, inner carpel facing the palea, and two distinct, shorter carpels on the side (fig. 16). The outer wall is usually six to eight cells thick, with the inner epidermis chlorophyllous. The base of the ovary contains one large vascular bundle which continues up the high, inner carpel, giving off first a branch to the ovule and then a branch to each style, and terminating before the apex of the ovary is reached.

Each ovary contains a single ovule attached directly to the inner carpel wall, no funiculus being present. The micropyle is directed downward and outward. The ovule is a modified campylotropous type and has two integuments, the outer one disintegrating after fertilization, whereas the inner one finally becomes adherent to the inner ovary wall. Each style arises from one of the outer carpels and divides into two feathery, stigmatic branches. Each branch is composed of four rows of elongated, nucleate cells, the distal ends of which are free and curved outward, thus forming a receptive surface for the pollen. The upper portion of the ovary bears simple hairs above the level at which the lodicules terminate.

ANTHESIS

Panicle primordia are initiated only in the year of anthesis. On May 1, 1941, at Ames, inflorescences were found to be near the ground level and only 1–1.5 cm. long. On May 4 the paleas, lemmas, and glumes were well-developed, and in some cases the lemmas were awned. Stamens and pistils were not in evidence.

Anthesis occurs late in May or early June at Ames, Iowa, although later, sporadic flowering is not uncommon. Anthesis takes place in mid-afternoon. Temperatures influence the rate of anthesis; the process can be hastened by holding mature spikelets in the hand. One floret opens each day in each normal spikelet; the duration of flowering for each spikelet is thus dependent upon the number of florets. Flowering of the panicle begins at or near the top and proceeds basally, but in each spikelet the process proceeds apically.

During the early stages of anthesis, the lodicules become turgid and force the palea and lemma apart. The filaments elongate greatly, and the three golden anthers tip over, usually one to one side and two to the opposite side, between the lemma and palea. The two stigmas "feather" out and one protrudes from each side of the lemma-palea opening. The distal end of the anther splits, and the dry pollen sifts out. On windy days, the anthers were seen to strike the stigmas of their own floret. The palea and lemma spring back somewhat after pollination.

CARYOPSIS

The so-called seed of commerce consists of the caryopsis, palea, lemma, and part of the rachilla. The true fruit or caryopsis is flattened, pointed apically, rounded basally, and has a tuft of hairs at its distal end. The ventral surface has a ridge, and the dorsal surface tends to be slightly concave. The caryopsis varies from 6–8 mm. in length, and measures up to 2 mm. in breadth, and almost 0.5 mm. in thickness.

EMBRYO

The embryo is situated at the base of the dorsal side of the caryopsis, and consists of the scutellum, the coleoptile, one foliage leaf, the stem apex, the radicle, and the coleorhiza. The embryo in soaked seeds is approximately 2 mm. long.

The median scutellar bundle branches at the scutellar plate and supplies the radicle, coleoptile, and shoot apex. The posterior or inner layer of the scutellum, in contact with the endosperm, consists of columnar epithelial cells. Most of the scutellar cells are rich in starch. The single foliage leaf has three large bundles and two smaller ones, the midrib being 90 degrees from each coleoptile bundle (fig. 17).

The radicle is approximately 0.2 mm. in length and tapers slightly toward its flattened distal end, which has a calyptrogen. The radicle is enclosed by the conical coleorhiza, the tissues of which merge with those of the scutellum. White hairs develop upon the coleorhiza soon after it has ruptured the pericarp.

PRIMARY ROOT

Shortly after emergence from the coleorhiza, the primary root has three distinct zones, the epidermis, cortex, and stele. The epidermis and most of the cortex are composed of thin-walled cells, which ultimately disintegrate. The endodermis is a single layer of cells, having greatly thickened and laminated radial and inner tangential walls. Unlignified passage cells occur. The pericycle consists of a single layer of large, radially-elongated cells in the arc between the protoxylem points, and usually two layers of smaller cells elsewhere. There are commonly four of these smaller cells in a group, adjacent to the passage cells of the endodermis.

The vascular tissues have the radial arrangement characteristic of roots. The phloem is arranged in five or six strands, each containing three cells arranged in the shape of a triangle, with the apical cell abutting on the pericycle. This apical cell is four-sided, somewhat diamond-shaped. A phloem strand is derived from a single phloem initial which divides to form two cells, one of which divides again.

Protoxylem vessels are situated between the phloem strands, usually one or two occurring in each xylem arc. The six-sided vessels are smaller than the metaxylem vessels, and are in contact with the small cells of the pericycle. Almost in the exact center of the root there is most commonly one metaxylem vessel, which enlarges before the protoxylem but becomes lignified later (fig. 18).

In the basal portion of a 14-day-old root, all the stelar cells except the phloem were found to be lignified. At 33 days, the protoxylem and metaxylem vessels had developed tyloses, which suggests that the primary root is of little importance as a conductive organ by that time.

ADVENTITIOUS ROOTS

The first adventitious roots arise at the scutellar node, approximately 5 days after germination in the greenhouse. Other roots arise above the first whorl, and by the fortieth day their number and length greatly exceed those of the primary system. The adventitious roots form the permanent root system of the plant. The epidermis gives rise to root hairs a short distance from the tip. The cortex consists of six to nine layers of cells with prominent intercellular spaces. The cells of he cortical parenchyma are elliptical, except those nearest the endodermis, which are somewhat flatter (fig. 3). As the root matures, the cortical cells become flaccid, their walls interlock, and the hypodermis becomes lignified.

In the proximal portion of a 59-day-old adventitious root, the endodermis was found to be well lignified, and no passage cells were evident. The pericycle of the adventitious root, like that of the primary root, is composed of alternate groups of large and small cells. The small cells, opposite the protoxylem points, retain their protoplasm longer than do the large cells.

After the metaxylem vessels enlarge, but before the protoxylem has

become conspicuous, the phloem is recognizable. Each phloem group consists of three cells which develop in the same manner as in the primary root. The phloem strands are in contact with the larger cells of the pericycle and alternate with the protoxylem points. A representative cross-section showed six metaxylem vessels, sixteen phloem groups, and sixteen protoxylem points.

Adventitious roots differ from the primary root in the following details: (1) the adventitious roots are of post-embryonic origin; (2) they have more metaxylem vessels; (3) they have more protoxylem points; (4) they have more phloem groups; (5) the epidermis and cortex function longer; (6) they live more than one year; (7) many are larger in diameter; (8) they have a pith; (9) there are no passage cells in the endodermis.

FIRST INTERNODE

The first internode or "mesocotyl" lies between the scutellar and coleoptilar nodes, and its length increases with depth of planting. The anatomy of the first internode is different from that of the succeeding internodes (fig. 21). The epidermis encloses a broad cortex consisting of thin-walled parenchyma, limited internally by an endodermis and containing one bundle. The pericycle consists of a single layer of hexagonal cells. The vascular system of the stele is composed of four strands; two of the strands are endarch, each with a large group of collateral phloem, the other two strands are exarch, with radial phloem. The limits of this phloem are difficult to recognize. The pith becomes ruptured, forming a central cavity.

COLEOPTILE

The coleoptile contains two bundles, placed 180 degrees apart and each 90 degrees from the scutellar bundle (fig. 17). The two bundles converge toward the apex. Occasionally, the basal portion of the coleoptile has four bundles, but two of these soon terminate. The bundles pass out from the scutellar trace just above the level at which a trace passes into the radicle.

The phloem is on the dorsal side and consists of approximately twenty sieve tubes and companion cells. The xylem usually contains three elements on the ventral side of the bundle, with no clear distinction in size between protoxylem and metaxylem. The remaining coleoptile cells are thin-walled. The coleoptile has an anterior slit approximately 5 mm. from its apex. There is no bud in the axil of the coleoptile.

CULM

Mature culms vary in height from 30–140 cm. The internodes are solid when young but become hollow, the schizogenous cavity being produced by the rupture and collapse of the central parenchyma. The upper internodes are in general longer than the lower, the one below the rachis being the longest. The upper internode is thickest in the middle, whereas the lower internodes are thickest at the apex.

The nodes are composed of a solid diaphragm of parenchyma and numerous vascular bundles. The bundles lie parallel in the internodes but form an interconnecting network in the nodes. The culm itself is not thickened at the nodes.

The structure of the main portion of the culm (fig. 22) is very similar to that of its upper portion, the rachis. The epidermis is heavily lignified on all but its inner walls. The hypodermis is lignified, except for small islands of thin-walled cells which develop chloroplasts in the uncovered parts of the culm. There are two well-defined circles of vascular bundles.

The shoot apex, from which the culm differentiates, is a compact dome of cells, slightly higher than broad. The tunica consists of one or two layers of rectangular cells having dense cytoplasm and large, spherical or elliptical nuclei. Anticlinal cell division predominates. The central core of cells constitutes the corpus, the cells of which are more irregularly polygonal and are characterized by periclinal walls (fig. 23).

SHEATH

The sheath is circular in cross-section and slightly or not at all keeled (fig. 24). It is closed nearly to its summit, but on the side opposite the blade a short slit is present, caused in part by the pressure of the growing culm. Usually the sheath is glabrous, but occasionally those of the lowermost leaves are pubescent. Because of the silica in the outer epidermis, the sheath is scabrous. The base of the sheath is swollen near the node, forming a characteristic thickening at that point. The lower sheaths tend to overlap one another and, unlike the upper sheaths, are often longer than their corresponding internodes.

Stomates are numerous on the dorsal epidermis but are rare or absent on the ventral surface. The latter layer is smooth to the touch and silvery in appearance. On the upper part of the sheath, the dorsal epidermis is most strongly lignified on the outer wall. This lignification occurs after the bundle caps have been lignified. The cells of the dorsal epidermis are somewhat round in cross-section, unlike those of the ventral epidermis. Basally in the sheath, the dorsal epidermis is more heavily lignified, chloroplasts are not as plentiful in the mesophyll, and the ventral epidermal cells are more rectangular. Schizogenous cavities are common between the bundles.

Two types of bundles appear in the sheath. In the larger type, protoxylem is distinguishable from metaxylem. Another feature is a huge bundle cap on the dorsal side (fig. 25). The smaller type of bundle, alternating with the larger and sunken midway into the mesophyll, is encircled by parenchyma, and has only a few xylem elements, a larger number of phloem cells, and only a small bundle cap.

BLADE

The blade was found to vary from 3.5 mm. to 19 mm. in width and from 100 mm. to 400 mm. in length (fig. 2). It is convolute in the bud, and when young, the blades as well as the sheaths are purplish at the base.

Mature blades are flat, taper to a point, diverge at an angle from the stem, are slightly keeled below, almost ridgeless above, and are arranged distichously. The ligule at the junction of the blade and sheath varies in height from 0.5 to 2 mm. and is membranous, short, truncate, and lacerate at its summit. Auricles may be present on the young plants but were not seen on mature specimens.

All young leaves are pubescent on both surfaces, but mature ones usually are glabrous. The edges are scabrous because of the forward-pointing, one-celled, thick-walled spines, 58.8 to 64.7μ long. Each spine has one or two adjacent, short, thick-walled cells averaging about 17.6μ in length.

Stomates are more numerous on the upper surface of the leaf. There are generally two or three rows of stomates, with their long axes parallel with that of the midrib, separated by several rows of long cells. The narrow guard cells have thickenings on their internal faces, and the lumen and nucleus of each is dumbbell shaped. The two accessory cells surrounding the guard cells are wider and have large, elliptical nuclei. The stomates are sunken slightly below the epidermal surface and average 40–41μ in length and 23–24μ in width.

The upper epidermis consists of unspecialized epidermal cells, bulliform cells, stomates, and occasional hairs. The unspecialized epidermal cells lie in rows between the bulliform cells and over the vascular bundles. These cells are slightly narrower over the bundles, becoming progressively larger until they merge with the bulliform cells. In cross-section they are rounded or slightly elliptical, and the outer wall is heavily cuticularized (fig. 26). The large bulliform or "motor" cells occur in three to seven rows, extending the length of the upper surface between the bundles (fig. 26). Reduction of the turgor in the bulliform cells causes their collapse and the consequent rolling of the leaf. The one-celled, nucleate hairs are modified epidermal cells. The lower epidermal cells are of uniform diameter in cross-section, and are externally cuticularized. Stomates and hairs similar to those of the upper epidermis are present. There is no palisade layer. Some of the subepidermal cells are isodiametric, but most of them are irregular, and longitudinal sections show them to be deeply lobed.

Fourteen days after germination, only five bundles may be well developed in a leaf; in a leaf 33 days old, five to seven bundles are usually fully developed; the mature leaf may have twenty-seven or more bundles. The midrib bundle is the largest, and the bundles of the blade are alternately small and large in a definite pattern (fig. 26). The midrib bundle is distinglished by a large wedge-shaped mass of sclerenchyma on the keeled lower side of the midrib, and a large flatter cap on the upper side. The outer walls of the superficial sclerenchyma cells are rippled. A bundle may have sclerenchyma above or below or both, or sclerenchyma may be entirely lacking. The mature fibers in the bundle caps, as determined by maceration, average 11.7μ in width and range from 936 to 1,117μ

in length. Cell walls of the bundle cap become strongly lignified by the fifty-ninth day after germination.

PROPHYLLUM AND TILLER

The buds in the axils of the lower leaves develop tillers and rhizomes and are covered by a protective organ, the prophyllum. The prophyllum has two bundles, 180 degrees apart, like those of the coleoptile. The tillers make their appearance within 8 weeks after germination by breaking through the leaf sheath.

The first emergent leaves of the tillers are atypical in form, the blade being extraordinarily short or absent. This peculiarity has no counterpart in the development of the main shoot. The leaves of the tillers are placed at right angles to those of the main shoot, and the leaves of axillary shoots of a tiller are oriented at right angles to the leaves of the tiller.

RHIZOME

The rhizome is a lateral subterranean organ, aiding in the lateral expansion of the plant and being chiefly responsible for the production of the sodbound condition so characteristic of this species. Certain strains such as Parkland are designated as noncreeping, but they do have short rhizomes. Potted seedlings developed rhizomes about 6 months after germination.

The young rhizomes are white, becoming brown in maturity, and are covered by brown papery scales originating at nodes. The apex of the rhizome develops leaves which expand after the rhizome tip has come to the surface. Nodal buds also arise on the rhizome and subsequently become leafy shoots. The internodes on mature rhizomes averaged 11 mm. in length.

Near the distal end of the rhizome, only the protoxylem elements are lignified, but strong lignification of all xylem elements occurs as the rhizome matures. The tissue sytems of the mature rhizomes are shown diagrammatically in figure 28. Marked lignification is evident in the epipermis, one-layered hypodermis, the endodermis, and pericycle (fig. 29). The endodermis may be locally double.

Vascular bundles lie in contact with and somewhat confluent with the pericycle (fig. 4). A bundle usually consists of the bundle sheath, two to three protoxylem elements, two metaxylem vessels, xylem parenchyma, tracheids, sieve tubes, and companion cells. The lignification of the bundle sheath in the rhizome scales becomes completed before comparable cells in the rhizome have become lignified.

DISCUSSION

The development of the brome grass plant exhibits two phases. The first phase consists of the development of the seedling into a vegetative plant. The shoot apex remains at or near the ground level, but the leaves expand and tillers and rhizomes arise from buds in the axils of the basal

leaves. The adventitious root system assumes dominance over the primary root system, and the plant stores up food reserves.

In the second phase, the internodes elongate rapidly, the inflorescence develops, pollination occurs, and the fruit matures. In the second year, the tillers store up food, develop a root system, and the shoot apex of each tiller elongates rapidly. Shoots develop from the ends of the rhizomes in the first and succeeding years. Bonnett (12) and Evans and Grover (25) have noted much the same events in other grasses.

Comparison of the emergence of organs and anatomical features of brome grass with other grasses reveals some striking similarities and differences. The emergence of the primary root from the side of the coleorhiza in brome grass finds a counterpart in *Holcus sorghum*, as reported by Chi (20). Zinn (77) was of the opinion that this lateral emergence of the primary root is normal for grasses. In *Bromus inermis* the xylem of the primary root develops tyloses as early as the thirty-third day, impairing if not halting water conduction. Percival believed that in *Triticum* the primary root functions up to harvest time (52).

The restriction of the chlorenchyma to the lower half of the glumes, except in the region of the bundles, resembles the condition found in wheat (52). The enclosure of the entire, undifferentiated spikelet by the glumes in smooth brome grass is also characteristic of oats (33). The lemma is inserted on the rachilla and not on the flower stalk and therefore cannot be homologous with a perianth segment. The lodicules, on the other hand, are regarded as a reduced perianth. Hackel (32) homologized the awn of the lemma with the blade of the grass leaf. The part of the lemma above the insertion of the awn is the homologue of the ligule, and that part below is the homologue of the sheath. According to this view, lemmas of brome grass plants that lack an awn, lack the ancestral blade. The spreading of the lemma and palea in anthesis is caused by the swelling of the lodicules as in other grasses. Annular vessels occur in the tissues of the lodicules, a condition similar to that prevailing in *Zea mays* (68) and in other grasses (55).

Smooth brome grass resembles other grasses with respect to the insertion of one of its three stamens between the two lodicules. Stebler and Schröter (60) state that the anthers of grasses are versatile, whereas Bews (11) considers them basifixed. Lindley (44) defines versatile as being attached near the middle, whereby the two halves are nearly equally balanced and swing freely. By this definition, the anthers of smooth brome grass are basifixed. Annular vessels occur in the filament as in wheat and other grasses. The tapetal cells of *Bromus inermis* are binucleate at maturity as in *Agropyron repens* (46) and *Triticum vulgare* (52). Wodehouse (74) characterized the pollen grains of the Gramineae. *Bromus inermis* and *B. erectus* (23) pollen grains each contain two sperms and a tube nucleus, and conform to the general pattern. The sequence of events during anthesis is essentially as described for the species by Beddows (10).

The ovary is interpreted as being tricarpellate, in agreement with the

most widely accepted interpretation of the ovary of the grasses. The innermost layer of the ovary wall appears to be chlorophyllous as in *Poa pratensis* (1), and the vascularization of this organ is simple as Percival (52) found in wheat and Walker (66) found in *Bromus unioloides.*

Boyd and Avery (13), Merry (45), and others have discussed the homologies of the embryo of the grasses. The present writer regards the scutellum as the first leaf and the coleoptile as the second leaf. Both the scutellum and coleoptile are, therefore, greatly modified leaves. The embryo of *Bromus inermis* has no epiblast, a condition said by Bruns (16) to be characteristic of the genus.

Smooth brome grass has only one seminal root, as in timothy (24) and sorghum (19), in contrast with oats (33), which has from one to five. Numerous laterals, originating in the pericycle opposite the phloem, develop subsequently on the primary root as in wheat (52) and sorghum (20). Jeffrey (34) pointed out that the lateral roots of vascular plants originate in the pericycle, opposite the protoxylem points, except in the grasses.

The pericycle consists of both large and small cells. The latter become fully lignified later than the protoxylem, aiding in distinguishing between pericycle and protoxylem. In barley, Hector (33) designates as protoxylem certain cells that seem to occupy the position of the small pericycle cells of *Bromus.*

The phloem is similar in origin and structure to that of sorghum (20). Of the three cells of the protophloem group, the more peripheral cell is a sieve tube, a fact which Chauveaud (18) earlier recognized.

The presence of one or more metaxylem vessels in the center of the primary root is characteristic of grasses, occurring in brome grass, sorghum (20), wheat (52), and rice (33) among others. The expansion of the metaxylem vessels before the maturing of the protoxylem is common in grasses but is unusual for other vascular plants (34). In at least one particular, adventitious roots of brome grass resemble those of *Bouteloua curtipendula* (50), namely, in becoming the important absorptive system by the sixth week after germination.

There has been considerable controversy regarding the homologies of the coleoptile. Sargent and Arber, according to Avery (8), believe that the coleoptile represents two fused stipules, because it commonly has two vascular bundles. However, Percival (52) found two to six bundles in the coleoptile of wheat, and Avery (8) found two to five bundles in that of maize. The latter author (9) believes that this variation in the number of bundles indicates that the coleoptile is homologous with a foliage leaf. The anatomy of the coleoptile bundles in brome grass is very similar to that in wheat (52). Evans (24) prefers to use the term coleophyll (sheath-leaf), rather than coleoptile (sheath-feather).

The border parenchyma, which is a prominent feature of the leaf bundles of brome grass, is said by Lewton-Brain (43) and Carleton (17) to act as a transfusion tissue, delivering food from the mesophyll to the bundle. Directly inside the border parenchyma is an endodermis-like

sheath, also noted by Arber (5) in *Bromus hordeaceus*. The functional relationships of these two sheathing layers are not demonstrable on a purely morphological basis.

Smooth brome grass ligules are without vascularization, as is true of other grasses (5). Philipson (53) concluded that the ligule consists of the free, upper portion of the sheath and a median upgrowth of the adaxial epidermis of the leaf. Kennedy (38) believes the ligule to be a double sheathing axillary stipule. The prophyllum is regarded as a single, modified leaf by Arber (2) and by the present writer.

The histogen theory of Hanstein is not applicable to the shoot apex of smooth brome grass but the tunica-corpus theory of Buder and Schmidt, as restated by Foster (26) seems to be consistent with the observed facts.

Smooth brome grass has well-developed tillers before the eighth week. By way of comparison, Mueller (47) reported that *Bouteloua curtipendula, B. gracilis, Andropogon furcatus,* and *Panicum virgatum* tiller in 3 weeks and *Sorghastrum nutans* tillers in 4 weeks. The internodes on the rhizomes in smooth brome grass average 11 mm. in length whereas the internodes of *Andropogon scoparius* and *Panicum virgatum* are shorter (47). The rhizome scales in smooth brome grass have strong mechanical tissue.

Arber (3, 4) believes amphivasal bundles to be common in the rhizomes of the monocotyledons. Lauder-Thompson (42) found this type of bundle in the nodes of *Spartina townsendii,* and the present work has shown that such bundles occur in *Bromus inermis.*

SUMMARY

A study was made of the gross morphology, anatomy, and development of the smooth brome grass, *Bromus inermis* Leyss.

The radicle breaks out of the side of the white, pubescent coleorhiza. Tyloses developed by the thirty-third day, and the radicle was probably non-functional by that date.

The coleoptile was 10 mm. long by the fifth day, 20 mm. by the fourteenth day, and shredded off by the fifty-fourth day.

The first foliage leaf had emerged by the fifth day; the second leaf was 72 mm. long by the twenty-first day; three more leaves appeared before the fifty-fourth day and as many as ten leaves were present by the one-hundred eleventh day.

Tillers appeared before the fifty-fourth day and had typical leaves, as well as atypical leaves, the blades of the latter being very short or lacking. Rhizomes arose in about six months, emerging from the axils of the lower leaves.

Lateral roots, frequently in pairs, arise from the radicle. The first adventitious roots originate from the scutellar node, but subsequent roots arise from the coleoptilar and higher nodes.

The rachis is anatomically like the culm except that the rachis contains more chlorenchyma. Branches of the rachis contain numerous fiber cells.

A spikelet consists of a rachilla, two glumes, and two to ten florets. Both the first and second glumes have heavily lignified epidermal cell walls, and stomates occur in both the ventral and dorsal epidermis. The arrangement of bundles in the lemma is similar to that of the leaf. The outer walls of the epidermis of the palea are thickened, rippled, and contain stomates. Vascularization is present in the lodicules.

The ovary is tricarpellate and uniloculate. One vascular strand extends into the inner lobe, and branches into the ovule and into each style.

A mature pollen grain contains a tube nucleus, two sperms, and numerous starch grains.

The structure of the embryo conforms to the structure of the typical gramineous embryo, having a prominent scutellum, a radicle encased in a coleorhiza and a coleoptile enclosing the shoot apex. Only one foliage leaf primordium is present. No bud was found in the axil of the coleoptile.

The anatomy of the roots, first internode, culm, leaves, and rhizomes is also described in detail. No marked divergence from the characteristic pattern of the Gramineae was found.

PLATE I

Fig. 1. Panicles of two selections of *Bromus inermis*, approximately ¼ natural size.
2. Variation in width of blade of miscellaneous selections, approximately ¼ natural size.
3. Cross-section of the adventitious root. × 138.
4. Detail of a section of the rhizome. × 300.

PLATE I

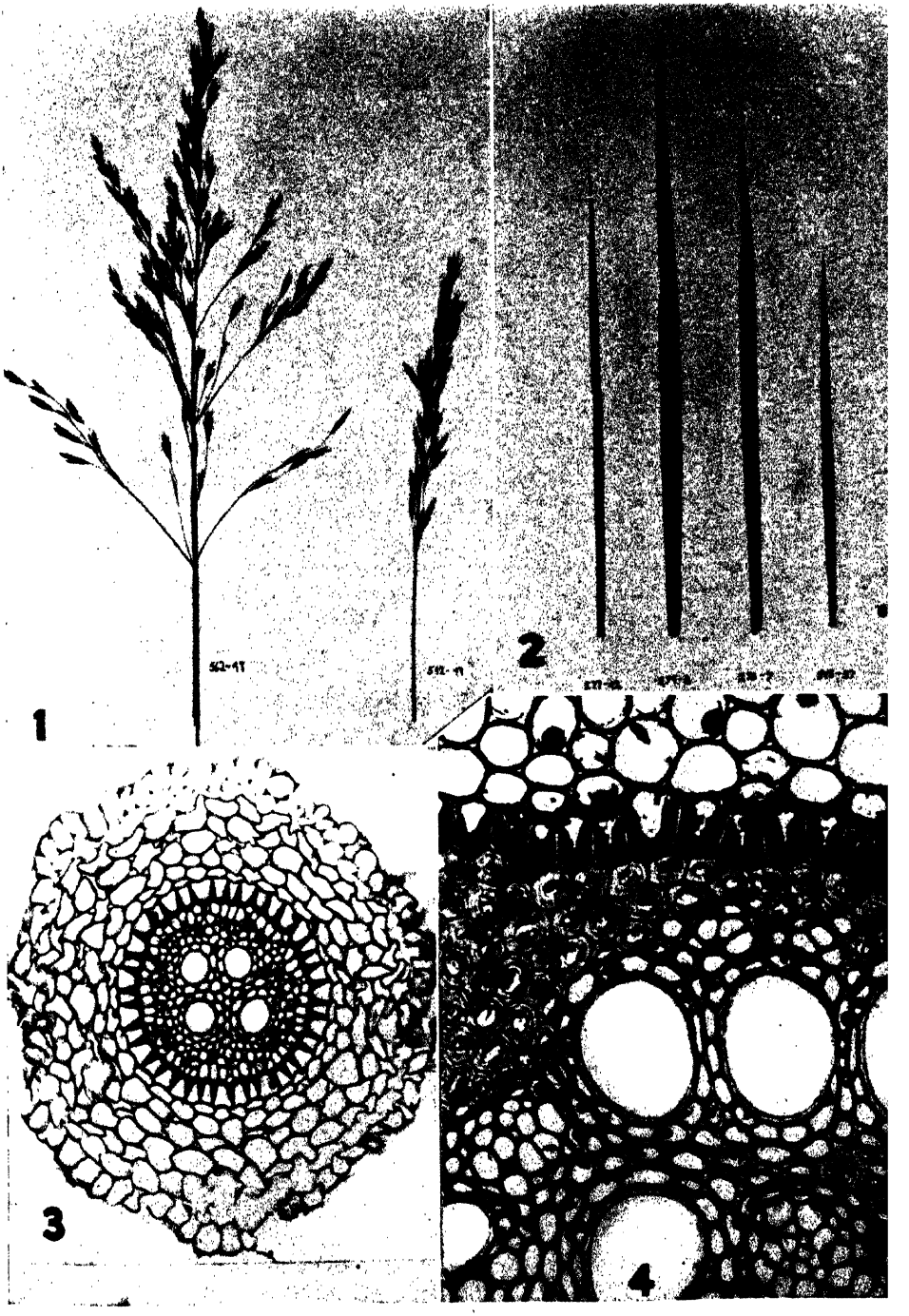


PLATE II

5. Detail of a section of the rachis. × 233.
6. Cross-section of a secondary branch of the rachis. × 133.
7. Outline of the second glume showing three vascular bundles. × 67.
8. Detail of a section of the second glume. × 300.
9. Detail of the first glume. × 233.
10. Outline of the first glume showing one bundle. × 100.
11. Detail of the lateral tip of the first glume. × 233.

PLATE II

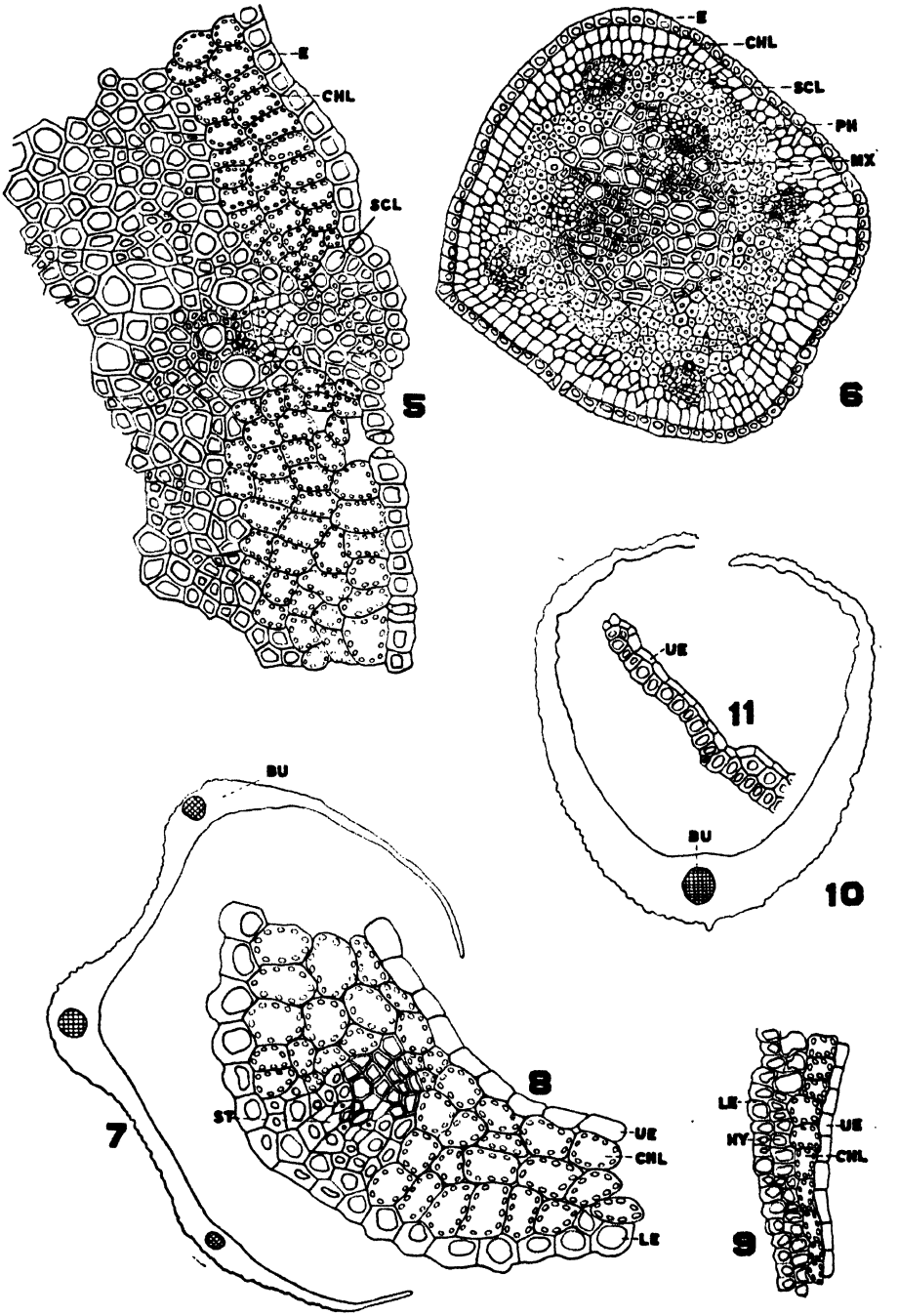
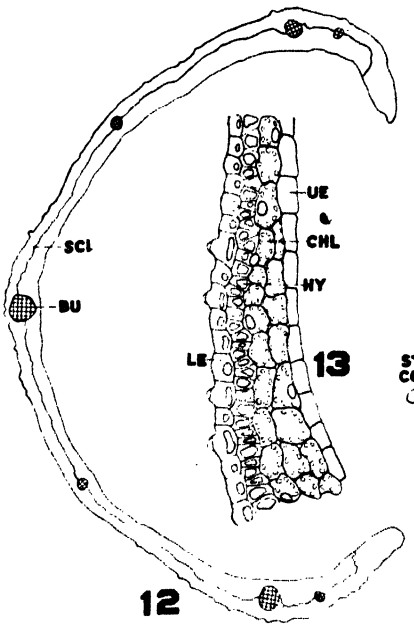
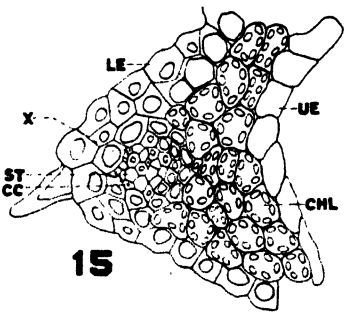

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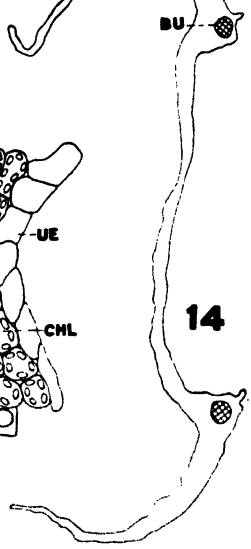
PLATE III

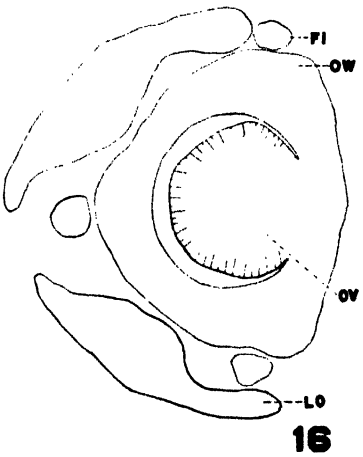
12. Outline of the lemma showing seven bundles. × 67.
13. Detail of a section of the lemma. × 233.
14. Outline of the palea showing a bundle in each keel. × 67.
15. Detail of a palea keel. × 300.
16. Cross-section of the ovary, lodicules, and filaments. × 67.
17. Cross-section of the embryo through the plumule, showing the position of vascular bundles in the scutellum, coleoptile, and leaf. × 100.

PLATE III


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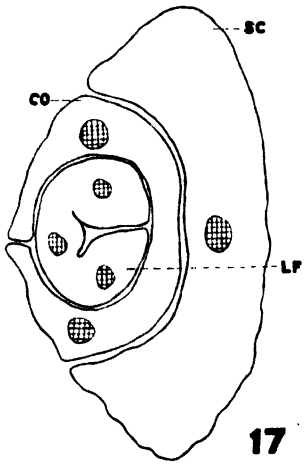

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PLATE IV

18. Cross-section of a mature primary root showing the disintegration of the cortex. × 200.
19. Cross-section of the stele of a young adventitious root. × 100.
20. Cross-section of the stele of an old adventitious root. × 200.
21. Cross-section of the first internode showing relative size and position of the stele and the cortical bundle. × 67.
22. Detail of a section of the culm. × 133.
23. Longitudinal section of the growing point of the stem. × 200.
24. Outline of a leaf sheath showing the extraordinary development of the bundle caps. × 27.

PLATE IV

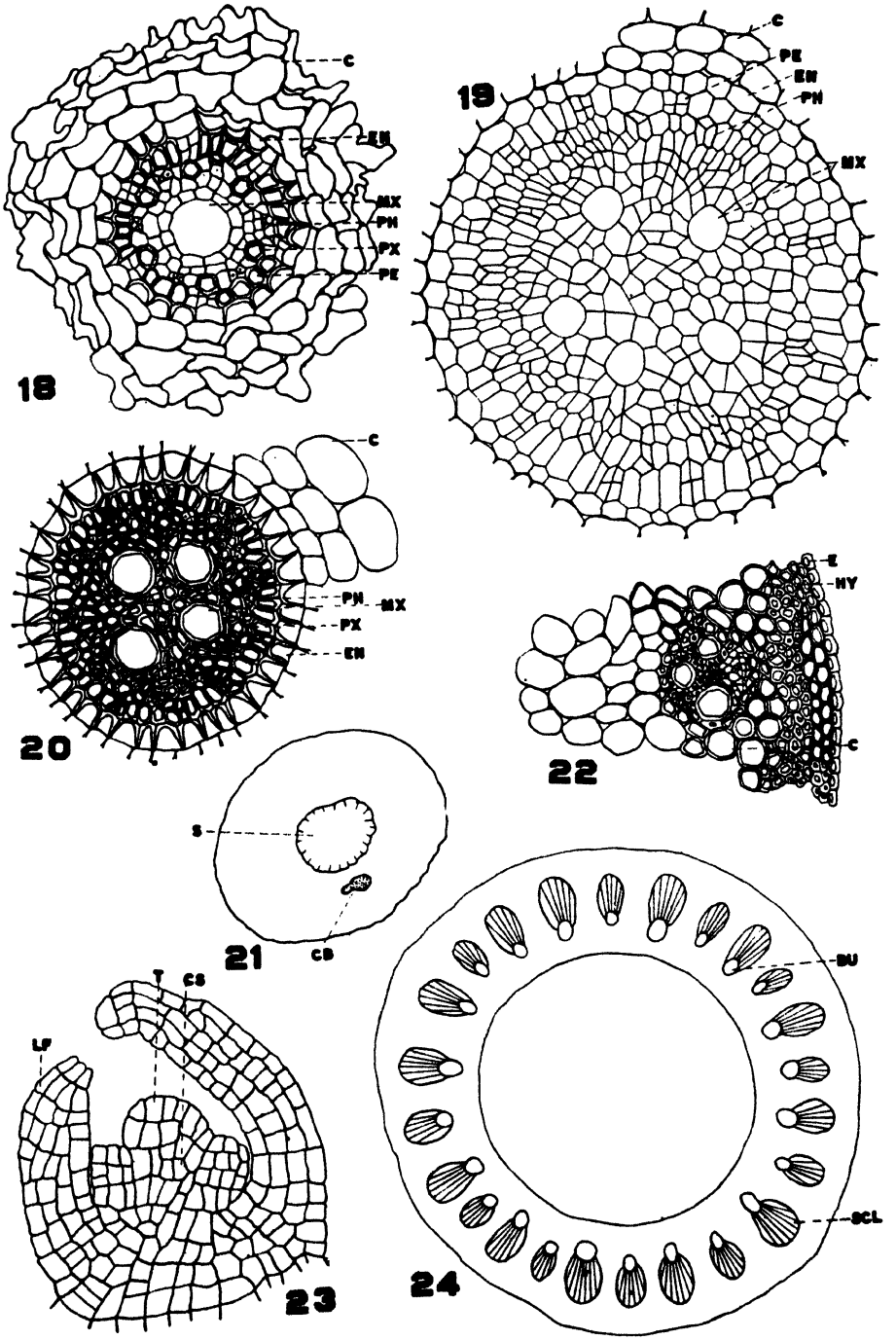

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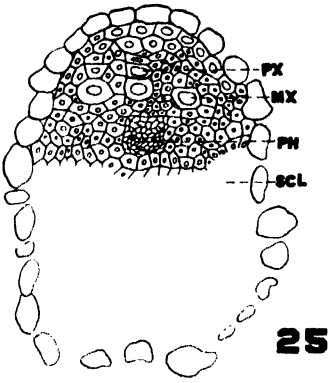
PLATE V

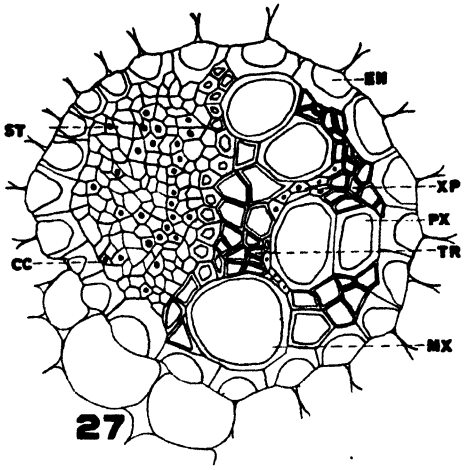
25. Detail of a sheath bundle, with most of the detail of the sclerenchyma omitted. × 133.
26. Outline of a leaf showing the relative position of the bundles and bulliform cells. × 14.
27. Detail of a leaf bundle. × 600.
28. Outline drawing of an old rhizome showing the position of the bundles. × 50.
29. Detail of a section of an old rhizome. × 233.

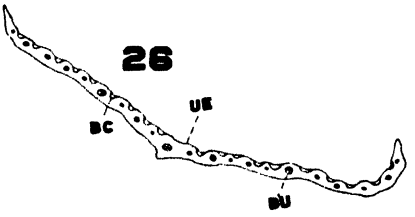
EXPLANATION OF ILLUSTRATIONS

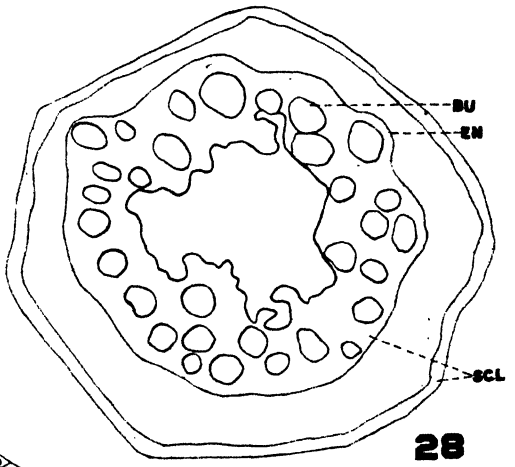
BC—bulliform cells
BU—Vascular bundle
C—cortex
CB—cortical bundle
CC—companion cell
CHL—chlorenchyma
CO—coleoptile
CS—corpus
E—epidermis
EN—endodermis
FI—filament
HY—hypodermis
LE—lower epidermis
LF—leaf
LO—lodicule
MX—metaxylem
OV—ovule
OW—ovary wall
PE—pericycle
PH—phloem
PX—protoxylem
S—stele
ST—sieve tube
SC—scutellum
SCL—sclerenchyma
T—tunica
TR—tracheid
UE—upper epidermis
X—xylem
XP—xylem parenchyma

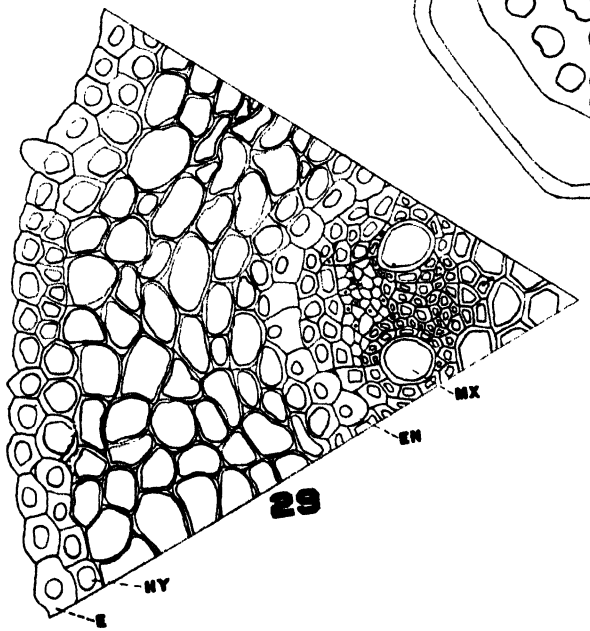
PLATE V


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CONCERNING AMERICAN RHOPALINI (HEMIPTERA, RHOPALIDAE)

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For several years I have been concerned in spare moments with a study of certain genera of the hemipterous family Rhopalidae.[1] Originally it was the intention to prepare monographic treatises of the different groups, but it early became apparent that for many reasons such could not be done and that the only practicable procedure was to issue reports of the studies from time to time. The present paper, the seventh in the series, is in continuation of these reports. It reviews the American species of Stictopleurus Stål and gives notes and illustrations[1] to aid in the recognition of the American genera of Rhopalini.

THE SPECIES OF STICTOPLEURUS

The genus Stictopleurus Stål is set apart from other genera of Rhopalini by reason of the characters of the metapleuron, pronotum, antennae, and genitalia. The body form, the vestiture, and the nature of the scutellum also are somewhat characteristic. The name Stictopleurus (= punctured pleuron) is descriptive of one of the distinctive features. The group is largely boreal and holarctic in distribution. As in other genera of the family the species vary much in color, size, and structural features.

There is a distinct need for a careful revisionary study in which particular attention is given to the genitalic characters. The type specimen of each described species must be re-examined. Data on the biology and ecology of some forms will need to be assembled and analyzed. Only by such an inclusive study can the true relationship of the American species to one-another and to those that occur in Asia and Europe be known. The following notes on the American forms will aid the investigator who is in a position to attempt such a study, and in the meantime, will dispose of some confusing points regarding synonymy and serve to aid workers to identify American specimens.

GENERIC CHARACTERS

Any attempt to redefine the genus without more exact knowledge of a greater number of extra-American species than I now possess must necessarily be of limited worth. However, an enumeration of those general features which together appear to set apart the species complex from other groups of species is offered below:

Form oblong, somewhat depressed; body not distinctly setose, the clothing hairs mostly in the nature of fine, soft, short, recumbent pubes-

[1] I am indebted to Mr. George Hopping for the figures in Plate I and to Mr. Judson McGuire and Dr. Reid Davis for those comprising Plate II.

cence. Punctures, especially of pronotum, coarse, close together, irregular, the interspaces somewhat rugulose. Antennal I surpassing head by approximately half its own length. Jugum narrow, produced anteriorly slightly beyond the truncate front end of lorum. Antennal shelf prominent, its apex forming a distinct antenniferous tubercule. Bucculae rather sharply declivent. First rostral segment surpassing bucculae by about half its own length. Pronotum (fig. 20) with front angles slightly swollen and somewhat prominent; the cicatrices on front lobe ending on each side in a more or less complete loop that surrounds or encloses a raised island; the collar punctate, separated from cicatrices by a smooth transverse ridge. Metapleuron not sharply divided into two parts, of about the same texture throughout, rather regularly coarsely punctate, the hind margin almost truncate and nearly perpendicular to the upper margin (fig. 9). Osteoles obsolete, the canals absent. Scutellum small, the base shorter than the sides, the latter sinuate, constricted distinctly behind the middle.

The male genitalia (figs. 1–4) show excellent specific characters, and also generic distinctness in that the general nature of the capsule and claspers is markedly different from that prevailing in other genera. In the past it has been extremely difficult and often impossible to establish the specific identity of female examples, but it now appears that the female genitalia offer worthwhile recognition features (figs. 15–18).

The type species of the genus (logotype, designatd by Oshanin in 1912) is *Stictopleurus crassicornis* (Linn.).

KEY TO SPECIES

1. Male 2
 Female 6
2. Genital clasper with a pronounced globose enlargement basally, strongly tapering distally, recurved so that the posterior face is concave (fig. 4) *punctiventris* (Dallas)
 Clasper, without sub-basal enlargement, flat or curved anteriorly 3
3. Clasper flat, tapering (fig. 1). General body color usually of a pronounced reddish hue *knighti* Harris
 Clasper curved anteriorly, the posterior surface convex. General color usually decidedly greenish 4
4. Clasper somewhat expanded and spoon-shape distally (fig. 2) *plutonius* (Baker)
 Distal part of clasper straplike, the sides more nearly parallel, the apex prominently, angularly emarginate 5
5. Size small (5.0–5.6 mm.). Clasper narrower. Upper lateral edge of genital capsule not produced (fig. 3) *viridicatus* (Uhler)
 Size larger (6.6–7.1 mm.). Clasper broader. Dorso-lateral edges of capsule somewhat produced *intermedia* (Baker)
6. Size large for the group (length, 7.2–8.6 mm.; width of abdomen, 3.1–3.6 mm.). Antennal I usually somewhat stouter than IV and only about twice as long as thick 7
 Size small (length 5.6–7.1 mm.; abdominal width, 2.2–2.7 mm.). Antennal I hardly as stout as IV and usually about 2½ times as long as thick 9
7. Opening at apex of venter exposing genital region, as viewed from the rear, broad, fully as wide as or wider than deep 8
 Opening exposing genital region not or scarcely broader than deep. Upper edge of urite IX not or slightly produced. Valves of ovipositer and urite beset with short spinules (fig. 15) *intermedia* (Baker)
8. Body stouter. Pronotum rather strongly declivent. Color not distinctly reddish, usually grayish-yellow with dark markings. Opening at apex of venter (fig. 16) more broadly U-shaped *punctiventris* (Dallas)

Body more depressed, the pronotum flatter. Color distinctly reddish. Opening at apex of venter, as seen from the rear, more distinctly V-shaped *knighti* Harris

9. Urites produced and sub-contiguous above the valves (fig. 17). Color often darker, the membrane then with fuscous streaks. Collar more sharply marked off. Vertex less arched. Pronotal punctures somewhat finer, less rugulose. Rostral I slightly shorter. *plutonius* (Baker)

Urites less strongly produced dorsally (fig. 18). Color usually more or less clear greenish yellow, with less pronounced dark markings. Collar less clearly delimited. Vertex more distinctly arched in front of ocelli. Pronotal punctures slightly more profound and rostral I faintly longer..... *viridicatus* (Uhler)

Stictopleurus punctiventris (Dallas)

1852 *Rhopalus punctiventris* Dallas, List of Hemip., 2:526.
1859 *Corizus novaeboracensis* Signoret, Ann. Soc. Ent. Fr., (3) 7:97.
1861 *Corizus borealis* Uhler, Proc. Acad. Nat. Sci. Phila., 12:284.
1872 *Corizus borealis* Uhler, Hayden's Survey Terr., Rept. for 1871, p. 403.
1876 *Corizus punctiventris* Uhler, Bull. U. S. Geol. Geog. Surv., 1:301.
1878 *Corizus punctiventris* Uhler, Bull. U. S. Geol. Geog. Surv., 4:505.
1885 *Corizus punctiventris* Provancher, Pet. Faune Ent. Can., 3:60.
1889 *Corizus punctiventris* Van Duzee, Can. Ent. 21:2.
1893 *Corizus puntiventris* Uhler, Proc. Ent. Soc. Wash., 2:370.
1894 *Corizus punctiventris* Uhler, Proc. Calif. Acad. Sci., (2) 4:237.
1895 *Corizus punctiventris* Gillette and Baker, Hemip. Colo., p. 21.
1904 *Corizus novaeboracensis* Van Duzee, 20th Rept. N. Y. St. Ent., p. 549.
1906 *Corizus punctiventris* Barber, Brooklyn Inst. Sci. Bul., 1:272.
1908 *Corizus crassicornis* Van Duzee, Can. Ent., 40:110.
1908 *Corizus crassicornis* Hambleton, Ann. Ent. Soc. Amer., 1:137, Pls. VIII & IX.
1908 *Corizus novaeboracensis* Baker, Can. Ent., 40:242.
1908 *Corizus novaeboracensis occidentalis* Baker, Can. Ent., 40.243.
1917 *Corizus crassicornis* Van Duzee, Cat. Hemip., p. 123.
1919 *Corizus crassicornis* Gibson, Can. Ent., 51:89.
1923 *Corizus crassicornis* Parshley, Hemip. Conn., p. 752
1928 *Corizus crassicornis* Blatchley, Heterop. E. N. Amer., p. 277.
1937 *Corizus punctiventris* Harris, Iowa St. Coll. Jour. Sci., 11:172.
1941 *Corizus crassicornis* Torre-Bueno, Ann. Ent. Soc. Amer., 34:285.
1941 *Corizus crassicornis* Torre-Bueno, Ent. America, 21 (NS):93.
1943 *Stictopleurus punctiventris* Harris, Iowa St. Coll. Jour. Sci., 17:203.

S. punctiventris varies much in size, general color, and markings. The descriptions by Hambleton (1908) and Blatchley (1928) are in general accurate and detailed, and are recent enough to preclude the need for a redescription here. Accurate determination is contingent upon a careful study of the genitalia. The male claspers (figs. 4 and 19) are distinctive. In the female the tip of the venter is more rounded as seen from the side (fig. 13) and is slightly emarginate at the mid-ventral line. The apical orifice exposing the genitalia is distinctly transverse, and the valves are proportionally shorter (fig. 16) than in related species.

SIZE: Length, male 6.5–7.0 mm.; female, 7.2–7.9 mm. Width, pronotum, 2.0–2.7 mm.

RANGE: *S. punctiventris* (Dallas) ranges across Canada and the northern part of the United States and southward along the mountain chains. I have examined specimens from the following states and provinces: Alberta, British Columbia, California, Colorado, Idaho, Indiana, Maine, Michigan, Minnesota, Montana, Nevada, New Hampshire, New York, Nova Scotia, Ohio, Ontario, Oregon, Pennsylvania, South Dakota, Utah, and Washington. The species is recorded in the literature, under

the names *crassicornis* and *borealis*, from Arizona, Massachusetts, Mexico, Nebraska, and New Mexico. Essentially nothing is known regarding its biology.

Comparison of the genitalia of specimens from the United States with specimens from Europe, and with Ribaut's excellent figures of the European species, several years ago disclosed that this widespread American form is not conspecific with *crassicornis* (L.), as had been held to be the case and led to the resuscitation for it of the name *punctiventris* Dallas. It is the commonest and most widely distributed member of the genus in America and had been described under several names. Uhler (1872) seems to have been the one first to suggest that his *Corizus borealis* might be synonymous with *Rhopalus punctiventris* Dallas. He noted, also, that it was close to *crassicornis* (Linn.) and called attention to the great variation in color within the species. Later (1876) Uhler synonymized *borealis* with *punctiventris*. I have seen the type of *borealis* (Uhl.), a male, from S. Colorado, in the collection of the U. S. National Museum, and can verify that it is the species here treated as *S. punctiventris*. Dallas' type of *punctiventris* presumably is in the British Museum of Natural History and has not been available for this study.

On the authority of Horvath, Van Duzee (1908) synonymized Signoret's *novaeboracensis* with *crassicornis* (L.), and Hambleton (1908) identified *novaeboracensis* with *punctiventris*, both of which names he considered as synonyms of *crassicornis* (Linn.).

Baker (1908) in his treatment of *novaeboracensis*, proposed several new names for what he called "the commoner forms of this species," Although he really gave no description, his key of less than a dozen lines appears to be sufficient to establish the names used. A few years back I made a search of the U. S. National Museum for Baker's specimens and, thanks to the splendid cooperation and expert help of Mr. Harry G. Barber am able to dispose of these names as follows:

Corizus novaeboracensis pallidus Baker. This name must stand as a synonym of *Stictopleurus viridicatus* (Uhler) and is discussed on a later page.

Corizus novaeboracensis intermedia Baker appears specifically distinct and is treated below as *Stictopleurus intermedia* (Baker).

Corizus novaeboracensis plutonius Baker is dealt with later as *Stictopleurus plutonius* (Baker).

Corizus novaeboracensis novaeboracensis Baker and *Corizus novaeboracensis occidentalis*, as represented by specimens from Colorado, in the U. S. National Museum and bearing Baker's determination labels, are outright synonyms of *Stictopleurus punctiventris* (Dallas).

Stictopleurus knighti Harris

1942 *Stictopleurus knighti* Harris, Jour. Kan. Ent. Soc., 15:100.

This splendid species is easily recognized by the genitalic characters (fig. 1). In general it is more roseate than *punctiventris* and has a

slightly different facies. The original description is sufficiently recent that it need not be repeated here.

SIZE: Length, male, 6.6–7.0 mm.; female, 7.6–7.9 mm. Width, pronotum 2.08–2.38 mm.

RANGE: Heretofore the species has been known only from Minnesota, but I now can record a female specimen from Thompson, Michigan, August, 1937, and a male, Agricultural College, Michigan, April 28, 1892, P. R. Uhler collection. The right paramere of this latter individual is atypical, apparently having been injured in development.

Stictopleurus viridicatus (Uhler)

1872 *Corizus viridicatus* Uhler, Hayden's Survey Terr., Report for 1871, p. 404.
1876 *Corizus hyalinus* Uhler, Bull. U. S. Geol. Geog. Surv., 5 (2nd Series):300 (in part).
1877 *Corizus hyalinus* Uhler, Bull. U. S. Geol. Geog. Survey, 3:407.
1908 *Corizus viridicatus* Horvath, Ann. Mus. Natl. Hung., 6:556.
1908 *Corizus viridicatus* Hambleton, Ann. Ent. Soc. Amer., 1:138.
1908 *Corizus novaeboracensis pallidus* Baker, Can. Ent., 40:243.
1914 *Corizus viridicatus* Barber, Jour. N. Y. Ent. Soc., 22:171.
1919 *Corizus viridicatus* Gibson, Can Ent., 40:89.
1928 *Corizus viridicatus* Blatchley, Heterop. E. N. Amer., p_0 277.
1941 *Corizus viridicatus* Torre-Bueno, Ann. Ent. Soc. Amer., 34:285.
1941 *Corizus viridicatus* Torre-Bueno, Ent. Amer., 21(NS):94.

This species was described from specimens collected in Colorado, Nebraska, and Dakota. A careful analysis of the original description leads one to believe that the type series must have included specimens of the species *viridicatus* and also pale individuals of the older *Liorhyssus hyalinus* (Fabr.). This suspicion is intensified by the fact that Uhler himself, in 1876, discarded the name *viridicatus* in favor of *hyalinus* and the following year spoke of *viridicatus* as a variety of *hyalinus*. Certain features set forth in Uhler's description, however, make it clear that he had before him some individuals of the species now recognized as *viridicatus*, e.g.: "front of face rather blunt. Apical joint of antennae rather slender, hardly longer than preceding. Posterior flap of metapleura oblique truncated, with the upper angles rounded at tip, and together with acetabula caps minutely punctured. Lateral edge of scutellum recurved, the tip sunken, and its apex almost acute." Hambleton (1908) and Horvath (1908), working separately, established the fact that *viridicatus* Uhler is specifically distinct from *hyalinus* (Fabr.). These workers correctly called attention to the kinship between *viridicatus* and *punctiventris* (Dallas), and Horvath pointed out that *hyalinus* is referable to Liorhyssus while *viridicatus* is a true Stictopleurus. In the U. S. National Museum collection is a female individual from Colorado, labelled in Uhler's handwriting "*Corizus viridicatus* Uhler, Type." In my opinion this individual is the female of the species here recognized. Also in the museum there are specimens bearing Baker's identification labels "*C. novaeboracensis* var. *viridicatus*." These latter unquestionably represent the "smaller, pale greenish, western form" for which Baker proposed the name *Corizus novaeboracensis pallidus*. There appears to be in the

museum no specimen bearing Baker's label "pallidus," a name which is preoccupied in the genus by Sahlberg's 1878 usage for a Siberian species.

As in other species of the genus, *viridicatus* varies much in size and in the amount and intensity of dark markings. For the general picture one may refer to the descriptions by Uhler, Hambleton, and Blatchley. The species is distinctly smaller than *S. punctiventris* (Dallas). The vertex is not so flat, the pronotum has less distinct impressions within the humeri and finer, more even punctations, the basal and apical antennal segments are less incrassate, and the basal rostral segment is proportionately slightly longer. The male clasper and genital segment are characteristic (fig. 3). In the female the shape of the apical abdominal segment, as seen from the side and from beneath, and the characters of the enclosed genital segments (fig. 18) are noticeably different from those of *S. punctiventris*. The species is very close to *S. plutonius* and separable from it only by close study.

SIZE: Length, male, 5.1–5.6 mm.; female, 5.6–6.8 mm. Width, pronotum, 2.0–2.3 mm.

RANGE: I have before me specimens of *S. viridicatus* from Alberta, Arizona, Colorado, Idaho, Iowa, Kansas, Minnesota, Montana, Nebraska, New Mexico, North Dakota, Saskatchewan, South Dakota, Utah, Washington, and Wyoming. The species has been recorded in the literature from California and District of Columbia. The latter record, based on a specimen in the Heidmann collection, appears extralimital and needs confirmation.

S. viridicatus is very close to *S. nysioides* (Reuter) from Siberia as represented by two female examples kindly sent me some years ago by Dr. A. N. Kiritshenko. A careful comparison of the male genital characters is needed to make clear the relation between these two species.

Stictopleurus plutonius (Baker)

1908 *Corizus novaeboracensis plutonius* Baker, Can. Ent., 40:243.
1944 *Stictopleurus plutonius* Harris and Shull, Ia. St. Coll. Jour. Sci., 18:202.

Closely related to *S. viridicatus* (Uhler) and heretofore confused with it and with *S. punctiventris* (Dallas), but recognizable by the genital characters.

Size, form, and vestiture about as in *viridicatus* (Uhler), punctation perhaps a bit coarser and more rugose. Color variable as in *viridicatus*, but often more conspicuously marked with black. Head noticeably declivent in front, the vertex and frons more definitely rounded above than in *punctiventris*, but less so than in *viridicatus*. Antennae with segment I greatly surpassing tylus, only slightly swollen, not stouter than IV; proportion of segments 11 : 22 : 22 : 25. Antenniferous tubercules from above slightly shorter and more obtuse than in *viridicatus*. Bucculae scarcely enclosing basal half of first rostral segment. Rostrum attaining metasternum; segment I just reaching prosternum, faintly shorter than in *viridicatus* (Uhler). Male with upper edge of genital segment more angularly produced than in *viridicatus*, the clasper larger, its apex broader and

somewhat spoon-shaped with the convex side to the rear (fig. 2). Female genitalia narrow as in *viridicatus*, the valves higher, the upper angle of the ninth urites produced and subcontiguous above the valves (fig. 17).

SIZE: Length, male, 5.0–5.5 mm.; female, 5.6–7.1 mm. Width, pronotum, 2.4–2.9 mm.

RANGE: I have seen examples from Colorado, Idaho, Nevada, Oregon, Utah, Washington, and Wyoming.

There are in the National Museum collections, apparently, no specimens of *plutonius* determined as such by Baker. However, there is a male from Colorado bearing Baker's label, "*Corizus novaeboracensis* var. *niger* Baker." It becomes obvious when one studies this specimen and others of the larger, more melanistic examples of this species that it must represent his *plutonius*, and that for some reason the manuscript name *niger* was discarded in favor of *plutonius*. The species is very closely related to *viridicatus*. In general it is more northern in distribution, and to judge from the many examples I have seen, is the more abundant of the two forms in Idaho and Washington, while *viridicatus* is dominant in Colorado and New Mexico.

Host plant records and life-history notes will be necessary to reveal the true relationship of this form to *viridicatus*.

Stictopleurus intermedia (Baker)

1908 *Corizus novaeboracensis intermedia* Baker, Can. Ent., 40:243.

The name *intermedia* was given by Baker to a series of specimens from Ormsby Co., Nevada, collected in July, and set apart because of their pale brown color and yellow scutellum. Examples from this series of individuals are present in the University of Kansas Snow Collection and in the U. S. National Museum collection, some of the latter bearing Baker's determination labels. Heretofore, the form has been considered as no more than a color variation of *punctiventris* (Dallas), but study of the genitalia discloses that there are rather constant differences in both male and female individuals. In many ways the form appears intermediate between *punctiventris* and *viridicatus*, and it will remain for the future to disclose the true relationship of these forms.

Size and shape about as in *punctiventris*, the body perhaps slightly more nearly parallel-sided. Color variable as in the other species, in pale examples the scutellum and connexivum often immaculate, melanistic examples with strongly maculate connexivum not uncommon, however. Pronotum with median line and lateral edges paler. Male genital segment somewhat as in *punctiventris*, the clasper more nearly like that of *viridicatus*. Female venter narrower (more laterally compressed) at the apex than in *punctiventris*, genital segments with the urites much less strongly produced and distinctly spinulose (fig. 15).

SIZE: Length, male, 6.7–7.1 mm.; female, 7.5–8.1 mm. Width, pronotum, 2.0–2.6 mm.

RANGE: Forty-four specimens are at hand from Colorado, Montana, Nevada, Oregon, Utah, and Washington.

MISCELLANEOUS NOTES ON RHOPALINI

Torre-Bueno (Ann. Ent. Soc. Amer., 34:284-288, 1941) has contributed a splendid word picture of the state the taxonomist finds himself in when attempting to deal with members of this group. Harris reviewed the history of the family name (Jour. Kan. Ent. Soc., 15:63-64, 1942) and presented keys for the separation of the tribes and genera together with comments on their synonymy and validity (Ia. St. Coll. Jour. Sci., 17:197-204, 1943). The following notes and the illustrations are presented as further contributions toward a clarification of the relationships between these groups.

Liorhyssus Stål: The male genitalia and the female abdominal segments in the species of Liorhyssus known to me are of the same general nature, but show specific differences. The type of male genitalia is sufficiently different from that found in other groups of species of Rhopalini as to lend strong support for the thesis that Liorhyssus should be accorded generic status. Figure 8 portrays the genital capsule of *L. hyalinus* (F.) as viewed from the rear; while figures 12 and 21 show the key characters of the metapleuron and pronotum respectively.

Niesthrea Spinola: As in Stictopleurus and Liorhyssus the genital characters in Niesthrea show specific differences but are of the same generic type. This is evident from a comparison of the genital capsules of *N. sidae* (Fabr.) (fig. 7) and *N. pictipes* Stål. (fig. 6) with those of species of other genera. *N. pictipes* (Stål) clearly deserves specific identity and is here resurrected from synonymy. Numerous other valid species, many now sunken in synonymy, occur in the tropics.

Arhyssus Stål: The American species of Arhyssus appear to fall into two groups centered around *A. bohemanii* (Sign.) and *A. scutatus* Stål, respectively. Elsewhere I have shown that the *scutatus* complex is composed of several distinct species (Jour. Kan. Ent. Soc. 15:100-105, 1942). Figures 10 and 11 show the metapleura of *A. barberi* Harris and *A. parvicornis* (Sign.), respectively. Figure 22 pictures the pronotal characters of *A. barberi;* and the genital structures of *A. lateralis* (Say) are portrayed in figure 5.

PLATE I. MALE GENITAL CAPSULES

1. *Stictopleurus knighti* Harris.
2. *Stictopleurus plutonius* (Baker).
3. *Stictopleurus viridicatus* (Uhler)
4. *Stictopleurus punctiventris* (Dallas).
5. *Arhyssus lateralis* (Say).
6. *Niesthrea pictipes* (Stål).
7. *Niesthrea sidae* (Fabr.).
8. *Liorhyssus hyalinus* (Fabr.).

PLATE I

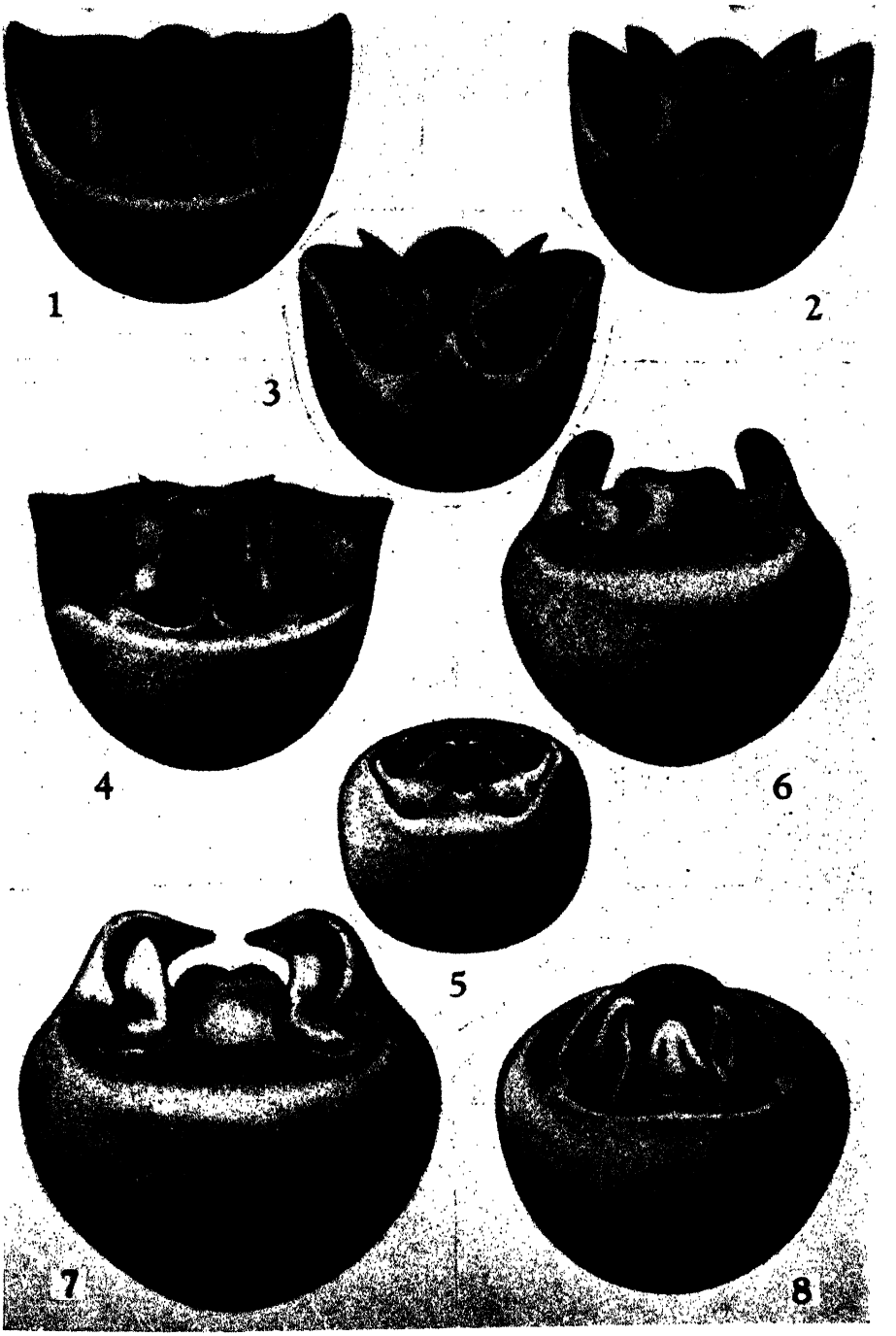
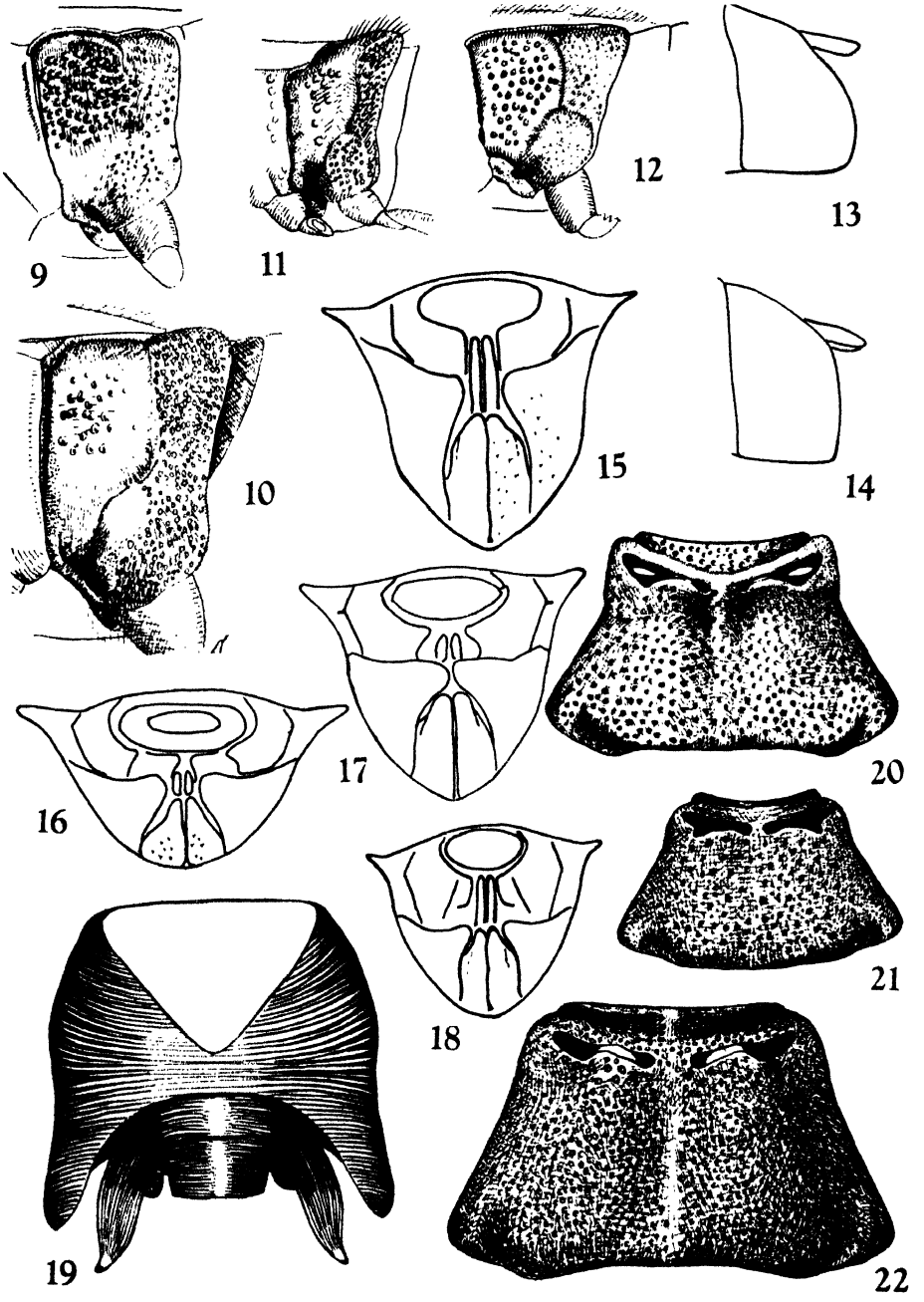


PLATE II. KEY CHARACTERS OF AMERICAN RHOPALINI

9. Metapleuron of *Stictopleurus punctiventris* (Dallas).
10. Metapleuron of *Arhyssus barberi* Harris.
11. Metapleuron of *Arhyssus parvicornis* (Sign.).
12. Metapleuron of *Liorhyssus hyalinus* (Fabr.).
13. Tip to venter, lateral aspect, of female *S. punctiventris*.
14. Tip of venter, lateral aspect, of female *S. plutonius*.
15. Female genital segments, caudal aspect, *S. intermedia* (Baker).
16. Female genital segments, *S. punctiventris* (Dallas).
17. Female genital segments, *S. plutonius* (Baker).
18. Female genital segments, *S. viridicatus* (Uhler).
19. Male genital capsule, dorsal aspect, *S. punctiventris*.
20. Pronotum, *S. punctiventris* (Dallas).
21. Pronotum, *Liorhysus hyalinus* (Fabr.).
22. Pronotum, *Arhyssus barberi* Harris.

PLATE II



A SECOND SUPPLEMENT TO THE CATALOGUE OF IOWA PLANTS IN THE IOWA STATE COLLEGE HERBARIUM[1]

ADA HAYDEN

From the Botany and Plant Pathology Section, Iowa Agricultural Experiment Station[2], and the Department of Botany, Iowa State College.

Received March 17, 1944

The plants reported in this paper supplement the annotated list of Cratty published in 1933, and the contribution of Augustine (1940) and 1941), Brown and Brown (1939), Fults (1934), Goodman (1939), Goodman and Leyendecker (1942), Hayden (1940 and 1943), Kornfeld (1942), Leyendecker (1941), Paris (1940), Sons (1941), and Tolstead (1938). They consist, with a few exceptions indicated in the text, of species and varieties not previously listed for the state; some occur in other herbaria. In some cases specimens referred to in this list have been incorrectly designated in the herbarium of Iowa State College as plants of a different range and identity. The numbers which appear in brackets are the accession numbers of Iowa State College Herbarium, not those of the collector.

Assistance received from Dr. Alan A. Beetle, Mrs. Agnes Chase, Dr. F. J. Hermann, Mr. E. J. Palmer, Dr. Lloyd H. Shinners, and Dr. Lyman B. Smith in the determination of specimens is gratefully acknowledged.

NAJADACEAE (Pondweed Family)

Potamogeton gramineus L. var. *maximus* Morong ex. Bennett
Grass-leaved Pondweed

See Rhodora 45: 149. 1943.

P. gramineus L. var. *maximus* Morong ex Bennett

P. gramineus L. var. *maximus* Morong

P. lonchites Tuckerman

EMMET Co., Armstrong, broad leaves where slough had dried, 1885, Cratty (86,975); sloughs, July, 1890, Cratty, July 11, 1897, Cratty (86,978) and (12,569) July 11, 1897; marshes, July 1 and Aug. 21, 1897, Cratty (106,547, 73,533, and 86,977) July 11 and Aug. 21, 1897. Eleven of the nineteen sheets in the herbarium seem representative of the typical variety of *P. gramineus* and eight represent the variety *maximus*.

Potamogeton panormitanus Biv. var. *major* Fern. Mem. Gray Herb. Harvard Univ. 3: 64. 1932. Wide-leaved Panormitanic Pondweed

DICKINSON Co., East Lake Okoboji, Aug. 1, 1896, Shimek (25,026); West Lake Okoboji, Aug. 3, 1897, Cratty (12,593).

[1] Journal Paper No. J-1192, of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 366.

[2] The Fish and Wildlife Service (U. S. Department of the Interior), Iowa State College, Iowa State Conservation Commission, and the American Wildlife Institute cooperating.

Potamogeton panormitanus Biv. var. *minor* Fern. Mem. Gray Herb. Harvard Univ. 3:64. 1932. Narrow-leaved Panormitanic Pondweed

CLAY Co., Lake Twp., Sec. 34. Submerged in two to three feet of open water on the northeast quarter of Round Lake, July 14, 1937, Hayden 10,120; DAVIS Co., Marion Twp. Abundant in Lake Wapello about six miles northwest of Drakesville, June 24, 1939, Hayden 9,642.

Both varieties of *P. panormitanus* have been designated as *P. pusillus* on herbarium sheets, before its relation to *P. panormitanus* was understood.

GRAMINEAE (Grass Family)

Alopecurus carolinianus Walt., Fl. Carol. 74. 1788. Annual Carolina Wild Timothy

See Am. Jour. Bot. 21:136. 1934.

A. ramosus Poir., in Lam., Encycl. 8:776. 1808.

A. geniculatus L. var. *ramosus* St. John. Rh. 19:167. 1917.

A. geniculatus of auth. not L.

APPANOOSE Co., Cincinnati, 1905, Wilson (48,916); Sedan, May 21, 1901, Pammel (8,802); BENTON Co., Vinton, June, 1911, Brant (95,938); DAVIS Co., June, 1919, Co. Agt. (96,575); DECATUR Co., Morgan Twp., June 3, 1902, Shimek (108,217); June 7, 1910, Anderson (53,402); EMMET Co., Armstrong, June 22, 1891 Cratty (87,146); IOWA Co., Marengo, May 27, 1919, Zentmire (96,335); JACKSON Co., Maquoketa, June 4, 1894, Shimek (5,533); KEOKUK Co., Sigourney, May 26, 1924, Allen (115,651); LEE Co., June 21, 1931, Fults 1,061; LINN Co., May 16, 1925, Sheets (117,754); MUSCATINE Co., Muscatine, May 23, 1922, Merrill (102,932); sand mounds south of Muscatine, 1935, Brown and Brown (146,417); POLK Co., Des Moines, Carver (5,331); Commerce, 1926, Pammel (126,448); STORY Co., Ames, July 17, 1930, Buchanan (82,950); alfalfa field, College Farm, June 2, 1921, Warnock (98,633); July, 1895, Rich and Gossard (5,530); TAYLOR Co., Lenox, introduced in straw mulch, June, 1919, Sealy (96,570); WARREN Co., June 20, 1921, Roring (107,006).

This annual plant has been mistaken for typical *Alopecurus geniculatus* L., which apparently does not reach Iowa.

Cenchrus longispinus (Hackel) Fernald. Rhodora 45:387. 1943. Sandbur

C. echinatus forma *longispinus* Hackel in Kneucker, Allg. Bot. Zeitschr. IX, 169 (1903).

C. echinatus of manuals.

C. tribuloides of manuals.

C. pauciflorus of manuals.

This plant occurs as a weed throughout Iowa in sandy soil. The specimens are referred to *C. tribuloides* L., which is now known to be the coarse southern coastal and tropical species, and *C. pauciflorus* Benth., which is a plant of Texan and Mexican distribution.

Andropogon virginicus L. Virginia Bluestem or Beardgrass

WAPELLO Co., Keokuk Twp., Sec. 34, eroding clay slopes of hills bordering woods along Soap Creek about two and one-half miles northwest of Floris, Nov. 16, 1941, Hayden 8,446. Infrequent.

Aristida curtissii (A. Gray) Nash Curtiss' Triple-Awn Grass

CHEROKEE Co., Cherokee, Sept. 5, 1920, Pammel (97,982); Pilot Twp., Sec. 22, growing in the soil-filled cracks of Pilot Rock, a large boulder located three miles south of Cherokee, Sept. 5, 1937, Hayden 7,083; HARDIN Co., Steamboat Rock, 1902, Shimek (108,198); LYON Co., Granite, Aug. 28, 1920, Pammel (98,599); Gitche Manito State Park, Sept. 3, 1931, Cratty (137,638); Gitchie Manito State Park, growing in cracks in the rock in little soil, also infrequent in deeper soils between the rock outcrops, Sept. 13, 1934, Jess Fults 2,942 and 2,943.

Elymus riparius Wiegand. Rhodora 20: 84-86. 1918. Riverbank Wild-Rye

ADAIR Co., Nodaway River, July 2, 1892, Stewart (7,113); ALLAMAKEE Co., two miles west of New Albin, Sept. 15, 1934, Tolstead (143,686), Parker (119,613); STORY Co., Ames, Carver (7,140); WEBSTER Co., Woodman's Hollow, two miles north of Dolliver State Park, Sept. 6, 1934, Fults 2,870; WINNEBAGO Co., Lake Mills, Aug. 21, 1919, Pammel (78,850).

Some specimens were reported under the names of *E. villosus*, *E. canadensis* var. *brachystachys*, *E. arkansanus*, and *E. canadensis*.

It was regarded by Wiegand as a near relative from which it differs in the following constant characters: the longer awns, more regularly exserted spikes and more generally villous leaves. Sons (1941) in a study of Iowa *Elymus* has brought together the Iowa specimens.

Panicum subvillosum Ashe Wiry Witch Grass

ALLAMAKEE Co., Iowa Twp., Sec. 28, about five miles southwest of New Albin, open dunes on the terrace of the Upper Iowa River Valley growing in large colonies on sand, June 18, 1940, Hayden 8,196.

This species is a plant of dry woods and sandy ground.

Panicum flexile (Gattinger) Scribner

PALO ALTO Co., Freedom Twp., Sec. 8, along the low, sandy shore of Medium Lake, Aug. 26, 1940, Hayden 8,220.

Throughout its distribution, it frequents sandy, mostly damp, soil in meadow or open woods.

Schizachne purpurascens (Torr.) Swallen False Melic

Melica striata Hitchc.

Melica purpurascens Hitchc.

Avena torreyi Nash.

DUBUQUE Co., Liberty Twp., Sec. 26, rocky woods in Pine Creek Hollow, two miles northwest of Luxemburg, June 17, 1941, Hayden 8,174;

WINNESHIEK Co., Decorah Twp., Sec. 6, growing in linden-maple woods, June 2, 1934, Tolstead (143,756).

CYPERACEAE (Sedge Family)

Carex annectens Bickn. Bull. Torrey Club 35: 492. 1908.
Yellow-fruited Sedge

C. vulpinoidea var. *ambigua* Barratt
C. vulpinoidea var. *platycarpa* Gay
C. xanthocarpa var. *annectans* Bickn.
C. setacea var. *ambigua* Fern.
C. vulpinoidea var. *annectans* Far.

DAVIS Co., Lick Creek Twp., Sec. 26, one mile west of Floris, in dry woods on the Hill Culture Experimental Farm, June 26, 1938, Hayden 10,908. A plant of wet fields or roadsides.

Carex laevivaginata (Kükenth.) Mackenzie Smooth-sheathed Sedge
In Britton and Brown Ill. Flora ed. 2, 1: 371. 1913.
See Rhodora 17: 231. 1915.
C. stipata Muhl. var. *laevivaginata* Kukenth.

JOHNSON Co., Iowa City, Hitchcock (2,317); LEE Co., Keokuk, June 1, 1897, Shimek 15. These specimens were separated from *C. stipata* by Charles Gilly. Fernald (1915) regards *C. stipata* as a plant of the Canadian and Transition Zones, but *C. laevivaginata* as essentially Alleghenian. A plant which grows in wet locations in the shade or open.

Scirpus americanus Pers. var. *polyphyllus* (Boeckl.). Beetle, Am. Jour. Bot. 30: 399. 1943.

BUENA VISTA Co., Storm Lake Twp., Sec. 4, on the northwest, sandy shore of Storm Lake, June 1, 1941, Hayden 8,283; CLAY Co., Lake Twp., Sec. 25, sandy soil margin of Mud Lake, Aug. 4, 1934, Hayden 148; Lake Twp., Sec. 25, gravelly shore at the edge of Mud Lake in Dewey's Pasture, May 31, 1936. Hayden 767 in part; Lake Twp., Sec. 25, margin of swamp, east side of Dewey's Pasture along a fence, June 22, 1936, Hayden 632; MONONA Co., Lincoln Twp., Sec. 35, on the west marshy border of Blue Lake in Lewis and Clark State Park, May 31, 1941, Hayden 8,324; PALO ALTO Co., Highland Twp., Sec. 33, marshy zone around cold springs outcropping on knolls in prairie five miles east of Ruthven south of the viaduct, May 15, 1936, Hayden 784.

Scirpus cyperinus var. *eriophorum* (Michx.) Kuntze. Rev. Gen. Pl. 2: 757. 1891. Wool Grass
Scirpus eriophorum Michx. Fl. Bor. Am. 1: 33. 1803.

DAVIS Co., Lick Creek Twp., Sec. 2, around water along the roadside about three miles northeast of Lick Creek, June 26, 1939, Hayden 9,196; Lick Creek Twp., Sec. 26, along the sandy course of Lick Creek on the southeast side of the Hillculture Experimental Farm one mile west of Floris, Sept. 7, 1940, Hayden 8,330.

The typical variety of *S. cyperinus* occurs in the northeastern quarter

of the State in Chickasaw, Clayton, Fayette, Delaware, Hancock, Winneshiek, and Worth Counties, also in the southeastern quarter, in Lee County; the variety *laxum* is represented in two eastern counties, Muscatine and Winnishiek; and the variety *eriophorum* in the southeast in Davis County.

Scirpus paludosus A. Nelson — Prairie Bulrush

See Am. Jour. Bot. 29: 82. 1942.

S. robustus var. *campestris* Fern.

S. robustus var. *paludosus* Fern.

S. campestris var. *paludosus* Fern.

PALO ALTO Co., Rush Lake Twp., on the moist, sandy bed of Rush Lake which was partly drained in the summer of 1942, July 23, 1942, Hayden 7,604. Beetle (1942) reports in his study of Scirpus collections representing only one county in Missouri, two counties from Minnesota, and three counties each in Kansas, Nebraska, North and South Dakota. Its habitats include fresh to strongly saline water.

Scirpus validus Vahl. var. *creber* Fernald. Rhodora 45: 283. 1943.
Northern Soft-stemmed Bulrush

ALLAMAKEE Co., Postville, July 8-11, 1904, Pammel, Orr, and Wilson (103,841); swamp, two miles south of Lansing, July 3, 1934, Tolstead (143,600); BOONE Co., Ledges, July 20, 1914, Ellis 67; CHICKASAW Co., Lawler, 1890, Rolfs (14,051); common in ponds, marshes and edge of streams, July 5, 1925, and Aug. 1, 1926, Spiker (125,770, and 127,193); CLAY Co., outlet of Lost Island Lake in six inches of water, June 22, 1936, Hayden 634; DICKINSON Co., E. Okoboji Lake, July 29, 1896, Cratty (88,243); DELAWARE Co., Delhi, June 25, 1926, Pammel (126,103); EMMET Co., Armstrong Grove, 1878, Cratty (88,246); July, 1923, Wolden (109,-381); FAYETTE Co., July, 1893, Fink (28,772); HANCOCK Co., Eagle Lake, near Garner, July 27, 1918, Pammel (96,119); JONES Co., Olin, July 1, 1898, Ball and Pike 2; LEE Co., infrequent in Wabash clay, Sec. 33, T. 68 N., R. 3 W., July 29, 1931, Fults 1,608; LINN Co., Cedar Rapids, Aug., 1902, Buchanan (82,084); LOUISA Co., Morning Sun, Carver, 1,314; LYON Co., Rock Rapids, July 6, 1896, Ball and Arilson 545; MONONA Co., in shallow water on the southwest marshy border of Blue Lake at Lewis and Clark State Park, May 31, 1941, Hayden 8,325; PALO ALTO Co., Highland Twp., Sec. 24, in a marsh fed by the outlet of a cold flowing spring, which emerges from a hillside, June 22, 1936, Hayden 646; SIOUX Co., Rock Valley, July 12, 1895, Jensen and Newell (20,065); WINNESHIEK Co., Decorah, July 14, 1899, Herbert Goddard (88,244).

Fernald (1943) states that *S. validus* Vahl., the plant of eastern tropical America, differs from the common plant of the United States and Canada, *S. validus* var. *creber* Fernald, in the following points: the variety *creber* has a more lax inflorescence, the backs of the scales are glabrous, the scale barely covering or when they are ripe not wholly covering the achenes; the perianth consists of usually coarser and rather

shorter bristles, which are copiously retrorse-setose; and the anther has a slender tip becoming prolonged.

Scirpus validus Vahl. var. *creber* Fernald forma *megastachyus* Fernald Rhodora 45:283. 1943. Long-spiked Soft-stemmed Bulrush

CLAY Co., Meadow Twp., Sec. 34, roadside northeast of Spencer, Aug. 23, 1935, Hayden 154; Lake Twp., Sec. 36, the dominant plant in a pond at the crossroads west of Lost Island Lake, Aug. 15, 1939, Hayden 9,195; HAMILTON Co., Ellsworth Twp., Sec. 9, in Little Wall Lake three miles south of Jewell, Aug. 16, 1936, Hayden 10,353.

The forma *megastachyus* is distinguished from var. *creber* by its prolonged-linear-cylindric spikelets up to 1.5 cm. long and achenes 1.7-2.5 mm. long by 1.5 mm. wide.

Scleria verticillata Muhl. Low Nut-rush

EMMET Co., Emmet Twp., Sec. 28, in bog, Aug. 27, 1929, Wolden (133,705); four miles northwest of Estherville in a hanging bog with *Ryncospora capillacea* and *Triglochin maritima*, Wolden and Hayden 220; EMMET Co., four miles north of Estherville, Sept. 11, 1934, Fults 2,919. This plant has not been located in similar habitats in northwestern Iowa.

Cyperus engelmanni Steud. Engelman's Cyperus

CLAY Co., Lake Twp., Sec. 26, along the sandy east shores of Trumbull Lake, Aug. 22, 1940, Hayden 8,348; EMMET Co., Iowa Lake, July, 1897, Pammel and Cratty (2,601); PALO ALTO Co., Highland Twp., Sec. 6, sandy shore of Lost Island Lake, Sept. 8, 1936, Hayden 697; WINNEBAGO Co., Lake Mills, Aug. 17, 1922, Pammel (105,933); peat bog plants of northern Iowa, Sept., 1908, Pammel (78,255).

LILIACEAE (Lily Family)

Maianthemum canadense Desf. var. *interius* Fernald. Rhodora 16:211. 1914. Western False or Wild Lily-of-the-Valley

CHICKASAW Co., Little Turkey River on river bank, May 17, 1926, Spiker (125,555); FAYETTE Co., Fayette, May, 1893, Fink (28,837); woods, Fayette, May 20, 1894, Fink (26,785). Moist wooded banks bordering Pine Lake in Pine Lake State Park at Eldora, May 15, 1941, Hayden 8,479; WINNESHIEK Co., Decorah, June 2, 1876, Holman (88,547); May 20, 1895, Herbert Goddard (71,590); on south hillside on moist, calcareous, rocky, humus soil in linden-maple woods; May 28, 1934, Tolstead (143,527).

Eight of the ten specimens occurring in the herbarium of Iowa State College have pilose stems, rachis and under side of leaves which characterize the plant of western distribution. Two specimens, one from Story County and one collected in Muscatine County, appear to be typical of the smooth variety.

FAGACEAE (Oak Family)

Quercus velutina Lam. var. *missouriensis* Sarg. Missouri Black Oak

WAPELLO Co., Keokuk Twp., Sec. 34, at edge of woods along Soap Creek, Oct. 1, 1938, Hayden 9,828.

Quercus bushii Sargent. Bot. Gaz. 65:453. 1918. Bush's Oak

Q. marilandica x *Q. velutina*

LEE Co., July 5, 1931, Fults 1,310; DAVIS Co., Keokuk Twp., Sec. 34; at edge of woods along Soap Creek, June 26, 1939, Hayden 9,836. Both parents grow in this vicinity but *Q. marilandica* is infrequent.

PORTULACACEAE (Purslane Family)

Talinum rugospermum Holzinger. Asa Gray Bull. 7:115. 1899.
Rough-seeded Flame Flower

See Torreya 28:94-95. 1899. Rhodora 30:205-206. 1926.

T. teretifolium of auth. not Pursh

ALLAMAKEE Co., Iowa Twp., Sec. 21, one thrifty colony was seen on the shifting sands of an open dune on the terrace of the upper Iowa River Valley, June 16, 1940, Hayden 9,901. This plant appears to have been undistinguished from *Talinum teretifolium* Pursh of Eastern range. *T. rugospermum* which has rough grayish seeds and no corm may be distinguished from *T. teretifolium* which has smooth, black, shiny seeds and a corm.

RANUNCULACEAE (Crowfoot Family)

Anemone riparia Fernald. Rhodora 1:51. 1899. Riverbank Anemone

DUBUQUE Co., Liberty Twp., along a rocky riverbank in Pine Hollow State Park, June 17, 1940, Hayden 8,702.

Anemone quinquefolia L. var. *interior* Fernald. Rhodora 37:260. 1935.
Inland Wood Anemone

ALLAMAKEE Co., two miles north of Lansing in upland oak woods, May 5, 1934, Tolstead (143,960); BOONE Co., Ledges, April 1, 1910, Bissell and Pammel (52,527); CHICKASAW Co., common in open woods and in shaded banks, May 8, 1926, Spiker (125,487); CLAY Co., Peterson, May 15, 1920, Pammel (98,572); CLAY Co., Peterson Twp., Sec. 33, wooded slopes along the Little Sioux River at Peterson in Wanata State Park, May 9, 1941, Hayden 8,695; DELAWARE Co., Devil's Backbone, May 10, 1919, Pammel (95,897); open wooded slope, May 7, 1939, Murley 659; EMMET Co., High Lake woods, May 17, 1917, and May, 1908, Wolden 229 and (89,432); FAYETTE Co., Fayette, May 2, 1889, Baker 10; HARDIN Co., Iowa Falls, May 6, 1905 (89,430); JOHNSON Co., floodplain woods in Lake Macbride State Park, April 8, 1938, Loufek 438; LINN Co., April 26, 1918, Whaley (138,492); STORY Co., rich woodland, Ames, April 25, 1887,

Beyer (112,721); WEBSTER Co., Ft. Dodge, Paige (130,423); WINNESHIEK Co., woods, Decorah, Goddard (72,264); on east hillside in open woods, May 10, 1934, Tolstead (143,959).

The inland plant is distinguished from the smooth eastern variety by stems, pedicels, peduncles clothed with spreading villous hairs.

CRUCIFERAE (Mustard Family)

Draba reptans Fernald var. *micrantha* (Nutt.) Fernald. Rhodora 36:368. 1934.

D. caroliniana Walt.

D. hispidula Michx.

CLAY Co., Peterson Twp., Sec. 31, gravelly roadside west of Peterson near the border of Clay County, May 19, 1941, Hayden (7,394); CLAY Co., Peterson Twp., Sec. 19, growing on gravelly hills in open spaces between grasses, chiefly *Bouteloua gracilis* and *B. hirsuta*, April 29, 1942, Hayden 7,624; DICKINSON Co., May 29, 1904, Fitzpatrick (89,808); FAYETTE Co., sandy soil, Fayette, July 7, 1894, Fink (31,539); O'BRIEN Co., Waterman Twp., Sec. 11, on gravelly hills in open spaces between *Bouteloua gracilis* and *B. hirsuta*, April 29, 1942, Hayden 7,626; PLYMOUTH Co., dry hills south of Westfield, May 29, 1938, Goodman 3,037; WINNESHIEK Co., Decorah, May 14, 1881, Holway (41,751); in field, Decorah, May 1, 1894, Goddard (71,734); on north side of hill in calcareous rocks, May 8, 1934, Tolstead (143,913).

This variety *micrantha* was seen growing with *D. reptans* on rocky exposed hills of Clay and O'Brien Counties. Considerable variation occurred in the hairiness of the mature leaves.

Rorippa islandica (Oeder ex. Murr.) Bourbas var. *hispida* Butters and Abbe. Rhodora 42:28. 1940. Hairy Marsh Grass

Roripa palustris (L.) Bess.

Radicula palustris (L.) Moench of manuals.

Roripa hispida (Desv.) Brit. Torrey Bot. Club Mem. 5:169. 1894.

Radicula palustris var. *hispida* (Desv.) Rab.

CLAY Co., Freeman Twp., Sec. 11, seedlings growing in muddy, low ground in an oatfield, April 15, 1935, Hayden 5,009; Lake Twp., Sec. 25, growing in the dry bed of Mud Lake on the north side, Sept. 8, 1936, Hayden 4,092; FAYETTE Co., Auburn, July 19, 1924, Pammel (114,950); FLOYD Co., Charles City, Sept. 20, 1918, Tuttle (94,114); LYON Co., Little Rock, July 1, 1897, Ball (5,030); MITCHELL Co., twenty-one miles east of Osage, July 28, 1912, Tuttle (33,068); MONONA Co., Turin, Sept. 8, 1894, Pammel (5,025); MUSCATINE Co., Muscatine, June 20, 1898, Ball (5,032); O'BRIEN Co., Waterman Twp., Sec. 11, floodplain of Waterman Creek, Sept. 4, 1941, Hayden 7,422; STORY Co., King (5,033); Ames, Ash Ave., July 7, 1922, Pammel (105,791); VAN BUREN Co., Lebanon, July 5, 1897, Sample 503; WEBSTER Co., Badger, July 4, 1920, Pammel (98,447).

The hairy variety is so far represented in eleven counties; the smooth, in twenty-two. There are one-third as many sheets of the hairy variety as of the smooth variety.

ROSACEAE (Rose Family)

Crataegus hannibalensis Palmer. Jour. Arnold Arboretum 16:353. 1935. The Hannibal Thorn

APPANOOSE Co., Bellaire Twp., about three miles west of Centerville, open woods, Sept. 25, 1940, Hayden 8,381; DAVIS Co., Lick Creek Twp., Sec. 2, three miles northwest of Floris, open woods in pasture bordering Soap Creek, Sept. 7, 1940, Hayden 8,382; DECATUR Co., High Point Twp., pasture along Highway 3, frequent, Sept. 25, 1940, Hayden 8,383; WAPELLO Co., Keokuk Twp., Sec. 19, hillsides bordering Soap Creek along Highway 63, Sept. 25, 1940, Hayden 8,384; WAYNE Co., Benton Twp., about five miles west of Corydon in open woodland pastures, Sept. 26, 1940, Hayden 8,380.

This species is found in open woodland especially in pastures. The fruit is cherry red to bluish-red. The tree is about eight feet high and round crowned. It resembles *C. crus-galli*. The Iowa range thus far lies in the southern tier of counties.

Malus soulardi Britton Soulard's Crab

PALO ALTO Co., Lost Island Twp., Sec. 33, along the roadside about one mile north of Lost Island Lake, Aug. 6, 1939, Hayden 9,478. Frequent along roadsides in Clay and Palo Alto Counties.

Rubus nefrens Bailey. Gentes Herbarium 1:239. 1925.

VAN BUREN Co., 1928, Aikman (156,071).

Rosa woodsii Lindl. Woods' Rose

HAMILTON Co., Boone Twp., Sec. 7, two colonies were found two miles south of Webster City along the C. & N. W. railroad track at whistling posts one mile apart, July 3, 1935, Hayden 11,525 and 11,524 and 9,452; MONONA Co., Lincoln Twp., Sec. 35, sandy soil along the border of Blue Lake about one-half mile north of the shelter at Lewis and Clark State Park, May 31, 1941, Hayden 8,524 and 8,524a; WOODBURY Co., Liberty Twp., in a pasture near the town of Salix south of Sioux City, July 25, 1938, Hayden 11,637.

Specimens previously listed in Iowa State College Herbarium were not *R. woodsii*. This species is perhaps the earliest blooming wild rose of Iowa. Specimens taken from the Webster City station and cultivated in the garden at Ames bloomed from May 22 to June 10 or 15. The flowers open several days to a week earlier than *R. blanda*.

LEGUMINOSAE (Bean Family)

Lespedeza nuttallii Darl. Nuttall's Bush Clover

DAVIS Co., Lick Creek Twp., Sec. 26, growing on an open gravelly

slope of the Hill Culture Farm, one mile west of Floris; infrequent, Sept. 5, 1940, Hayden 9,908.

This collection appears to be nearing the western border of the known range of the species.

Lespedeza repens (L.) Bart. Creeping Bush Clover

HENRY Co., near Mt. Pleasant, Sept. 1897, Mills (4,448); LEE Co., near Keokuk, 1891, Rolfs (4,444); MONROE Co., near Melrose, Sept. 15, 1922, Pammel (106,728); VAN BUREN Co., near Bonaparte, Sept. 17, 1924, Pammel (116,104).

L. repens closely resembles *L. violacea* (L.) Pers., but the two species may be distinguished by their habit, length of stipules, and relative length of banner to keel of the flower.

Tephrosia virginiana (L.) Pers. var. *holosericea* (Nutt.) T. & G.
Hairy Goat's Rue

ALLAMAKEE Co., Union City Twp., Sec. 36, sandy river terrace of the Upper Iowa River Valley five to seven miles southwest of New Albin, Sept. 13 and 14, 1937, Hayden 10,306 and 5,020; Iowa Twp., Sec. 21, growing on an open dune in the valley of the Upper Iowa River, June 18, 1940, Hayden 9,949; sandy river terrace on south hillside, Sept. 13, 1937, Tolstead (151,389); BUCHANAN Co., Rowley, sandy soil, June 26, 1902, Pammel (81,151); DELAWARE Co., Delhi, Oct. 14, 1922, Pammel and E. R. Harlan (105,873); LEE Co., Sec. 28, T 67 N R 5W, infrequent in sandy soil. July 23, 1931, Fults 1,546; MUSCATINE Co., sand, Muscatine Island, 1928, Aikman (156,046); sand dunes near Muscatine, Aug., 1933, I. E. Melhus and R. E. Buchanan (140,933); sand mounds south of Muscatine, summer 1935, Brown and Brown (147,147).

The variety *holosericea* is distinguished by the pubescent upper surface of its leaves from the smooth-leaved typical variety. The eleven sheets in the herbarium representing five counties belong to the hairy-leaved variety. The plants occurred on sandy river terraces, floodplains, or sand dunes.

OXALIDACEAE (Oxalis Family)

Oxalis stricta L. var. *piletocarpa* Wiegand. Rhodora 27:123. 1925.
Hairy-capsuled Upright Yellow Sorrel

APPANOOSE Co., Sharon Twp., Sec. 33, two miles east of Centerville, on a roadside bank along Highway 13, May 13, 1939, Hayden 9,653; DAVIS Co., Lick Creek Twp., Sec. 15, sandy alluvial soil, Hill Culture Experimental Farm one mile west of Floris, Oct. 1, 1938, Hayden 9,115; VAN BUREN Co., Vernon Twp., Sec. 31, about two miles north of Mt. Sterling, Sept. 22, 1941, Hayden 8,650; WAPELLO Co., Keokuk Twp., Sec. 11, dry, rocky slopes near Cliffland above the Des Moines River, June 25, 1939, Hayden 9,656.

EUPHORBIACEAE (Spurge Family)

Acalypha virginica L. Virginia Three-seeded Mercury
See Rhodora 29:197-198. 1927. and Rhodora 39:16. 1937.
A. dygyneia Raf.

APPANOOSE Co., Chariton Twp., four miles west and two miles south of Moravia, Oct. 28, 1939, Hayden 9,306; DAVIS Co., Lick Creek Twp., Sec. 26, one mile west of Floris on the Hill Culture Experimental Farm, Oct. 26, 1939, Hayden 9,305; Salt Creek Twp., Sec. 6, three miles northeast of Floris near Addsdale on a dry, rocky hillside, Oct. 7, 1939, Hayden 9,307; LEE Co., Jackson Twp., rocky hillsides, along the Mississippi River near Keokuk, Oct. 27, 1939, Hayden 9,308; VAN BUREN Co., Farmington, Sept. 9, 1930, Pammel (136,074); WAPELLO Co., Keokuk Twp., Sec. 11, rocky woods, along the Des Moines River near Cliffton, Oct. 1, 1938, Hayden 9,155.

Most of the material formerly listed in the Herbarium of Iowa State College is now known to be *A. rhomboidea* Raf.

VIOLACEAE (Violet Family)

Viola nephrophylla Greene Kidney-leaved Violet

CLAY Co., Logan Twp., Sec. 16, in sedge zones around hanging bogs occurring along the hills bordering Elk Creek, May 9, 1941, Hayden 7,499; PALO ALTO Co., Highland Twp., Sec. 24, five miles east of Ruthven in the sedge zone around a cold spring in prairie south of the viaduct over Highway 18, abundant locally, May 8, 1941, Hayden 7,498.

Anderson (1942) reports *V. nephrophylla* from wet grassy borders of prairie springs in Dickinson County.

Viola papilionacea × *V. pedatifida* Brainerd. Bull. Torr. Bot. Club 40:249-252. 1913.

PALO ALTO Co., Highland Twp., Sec. 14, growing on the grassy bank of the railroad track. *V. pedatifida* occurs on dry soil and *V. papilionacea* on moister soil nearby, May 10, 1941, Hayden 7,483.

Viola septentrionalis Greene Northern Blue Violet
See Violets of N. A. Vt. Exp. Sta. Bull. 224:44. 1921.
Violaceae of Iowa. Stud. in Nat. Hist. State Univ. Iowa 17:61. 1936.
V. macounii Greene
V. subviscosa Greene
V. fletcheri Greene
V. nesiota Greene

APPANOOSE Co., Union Twp., Sec. 28, open woods about one mile north of Unionville, April 22, 1938, Hayden 11,373; BOONE Co., Ledges, April 1, 1910, Bissell and Pammel (52,579); CHICKASAW Co., New Hampton, May 11, 1926, Spiker (120,906); CLAY Co., Lake Twp., Sec. 25, moist open grassland, Aug. 14, 1939, Hayden 9,296; EMMET Co., Estherville Twp., Sec. 12, low woods, Ft. Defiance State Park, June 20, 1939, Hayden 9,294; woods

and prairie, May, Cratty (15,605); DAVIS Co., Lick Creek Twp., Sec. 26, along the sandy floodplain of Lick Creek, June 25, 1939, Hayden 9,293, 9,295, and 11,377; MAHASKA Co., Scott Twp., Sec. 19, about ten miles west of Oskaloosa along the wooded slopes of the Des Moines River, April 22, 1938, Hayden 11,372; STORY Co., Ames, April 27, 1893, Ball (22,950); WARREN Co., Union Twp., Sec. 19, six miles east of Indianola on Highway 2, April 20, 1938, Hayden 9,172; WINNISHIEK Co., July 9, 1914, Goddard (71,914); WEBSTER Co., Ft. Dodge, Paige (131,046).

Viola septentrionalis Poir. is distinguished from *V. sororia* Willd. by the sepals, usually ciliate to the tip, the blunt tip of the leaf. This species has been referred to *V. cucullata* Ait., which does not reach Iowa.

EBENACEAE (Ebony Family)

Diospyros virginiana L. var. *pubescens* (Pursh). Dippel, Handb. Laubholzk. 1:306. 1889. Pubescent-leaved Persimmon

Diospyros pubescens Pursh

VAN BUREN Co., Henry Twp., Sec. 2, rocky slopes, on the east side of the Des Moines River above the town of Bentonsport. Colonies of young trees are springing up around the old ones, Sept. 24, 1940, Hayden 9,904. The typical variety which is smooth is most frequently found in Missouri, though the hairy variety is scattered in distribution and commonly occurs with the typical variety, according to Palmer and Steyermark.

OLEACEAE (Ash Family)

Fraxinus americana L. var. *juglandifolia* (Lam.) Rehder
Walnut-leaved White Ash

DAVIS Co., Lick Creek Twp., Sec. 6, about four miles northwest of Floris, not infrequent in this region. This specimen is from a young tree; Sept. 7, 1940, Hayden 9,764; FREMONT Co., Washington Twp., Sec. 29, five miles north and two miles west of Hamburg, in Waubonsie State Park, Sept. 19, 1940, Hayden 9,765 and 9,766. This variety is distinguished from the typical variety by the leaflets being more or less serrate or crenate-serrate, less lustrous above, more or less pubescent beneath and less glaucous.

Fraxinus pennsylvanica Marsh. var. *austini* Fernald. Rhodora 40:452. 1938. Austin's Ash

F. campestris Britton in part.

PALO ALTO Co., Silver Lake Twp., Sec. 20, six miles south of Ruthven on the rocky bank of Silver Lake bordering the road north of the bridge over Silver Creek; trees about 20 feet tall, pistillate and staminate, July 31, 1940, Hayden 9,760 and 9,761.

The young branchlets are velvety tomentose; leaf-rachises and lower surfaces of leaves are more or less fulvous pubescent; mature samaras

are 2.8 to 3.7 (rarely -4) cm. long; the body is 1 to 1.7 cm. long, the spatulate are 4 to 8 mm. broad; the leaflets are commonly toothed.

VERBENACEAE (Verbena Family)

× *Verbena engelmannii* Moldenke. Rev. Sudam. Bot. 4:18. 1937.
Engelmann's Verbena

V. hastata L. × *V. urticifolia* L.
Synonyms:
V. paniculata-urticaefolia Engelm. Am. Jour. Sci. 46:101. 1844.
V. hastata × *urticifolia* Blanchard, in herb.
V. hastata × *urticifolia* Eggert, in herb.
V. hastata × *urticaefolia* Norton, in herb.
V. urticaefolia × *hastata* Farwell, in herb.

CLAYTON Co., St. Olaf, Aug. 10, 1924, Pammel (115,923); HARDIN Co., Iowa Falls, Peck (17,302); JOHNSON Co., Iowa City, Aug. 6, 1925, Pammel 708; LEE Co., along roadside near Keokuk, July 27, Mitchell (115,868); MUSCATINE Co., Muscatine, July 20, 1919, Pammel, Kelso, and Harlan (95,044); STORY Co., Ames, Sept., 1909, Campbell (84,561).

This species and the following were cited by Moldenke (1937) in his monograph. They occur where the ranges of the parents overlap.

× *Verbena illicita* Moldenke. Rev. Sudam. Bot. 4:18. 1937.
V. stricta Vent. × *V. urticifolia* L.
Synonyms:
V. stricto × *urticaefolia* Engelm. Am. Jour. Sci. 46:101. 1844.
V. stricta × *urticaefolia* Mackenzie, in herb.
V. stricta × *urticaefolia* Pond, in herb.
V. stricta × *urticifolia* Britton, in herb.
V. stricta × *urticifolia* Eggert, in herb.
V. stricta × *urticifolia* Stevens, p.p., in herb.
V. urticifolia × *stricta* Eggert, in herb.

HARDIN Co., Steamboat Rock, sandy soil, Sept., 1912, McKibben (123,449); DES MOINES Co., a common weed in sandy soil, proposed State Park, Burlington, Aug. 8, 1925, Pammel 960.

× *Verbena meochina* Moldenke. Rev. Sudam. Bot. 4:19. 1937.
V. simplex Lehm. × *V. stricta* Vent.
Synonyms:
V. stricto-angustifolia Engelm. Am. Jour. Sci. 46:101. 1844.
V. angustifolia × *stricta* Kellogg, in herb.
V. angustifolia × *stricta* Rydberg, in herb.
V. stricta × *angustifolia* Eggert, in herb.

BLACK HAWK Co., Cedar Falls, Sept. 28, 1920, Pammel (98,936); HARDIN Co., Steamboat Rock, sandy soil, Sept., 1912, Pammel (51,974); JONES Co., Oxford Junction, July 25, 1919, Pammel (97,273); STORY Co., Ames, Sept. 8, 1894, Pammel (15,324); Ames, June 30, 1898, Ball (15,309).

× *Verbena perriana* Moldenke. Rev. Sudam. Bot. 4:19. 1937.
Perry's Verbena

V. bracteata Läg. and Rodr. × *V. urticifolia* L.
Synonyms:
V. bracteoso-urticaefolia Engelm. Am. Jour. Sci. 46:101. 1844.
V. bracteoso × *hastata* Webber. Trans. Acad. St. Louis 6:40. 1892.
V. bracteosa × *hastata* Rydb. Fl. Rocky Mts. 740. 1917.
V. bracteosa × *stricta* Britton, in herb.
V. bracteosa × *urticaefolia* Mackenzie, in herb.
V. bracteosa × *urticifolia* Eggert, in herb.
V. bracteoso-stricta Engelm., in herb.
V. hastata × *bracteosa* Rydb., in herb.
V. officinalis × *bracteosa* Barnes, in herb.
V. stricta × *bracteosa* A. S. Hitchcock, in herb.
V. stricta × *urticifolia* Stevens p.p., in herb.
V. stricto-bracteosa Engelm., in herb.
V. urticifolia × *bracteosa* Eggert, in herb.

BLACK HAWK Co., Cedar Falls, July 5, 1895, Carver (22,919); CLARKE Co., *Osceola*, Sept. 27, 1924, L. H. and H. E. Pammel (114,682); DECATUR Co., waste places, July 23, 1903, Anderson (52,109); EMMET Co., riverbank near Estherville, July 12, 1927, and Aug. 5, 1922, Wolden (130,461 and 105,155); FREMONT Co., Hamburg, Hitchcock (15,300); JOHNSON Co., woods near riverbank, Hitchcock (15,301); STORY Co., Ames, July 14, 1896, Carver (15,326); Collins, Aug. 8, 1929, Leonard (134,217).

× *Verbena rydbergii* Moldenke. Rev. Sudam. Bot. 4:19. 1937.
Rydberg's Verbena

V. hastata × *V. stricta* Vent.
Synonyms:
V. stricto-paniculata Engelm. Am. Jour. Sci. 46:101. 1844.
V. hastata × *stricta* Rydb. Bot. Surv. Nebr. 3:18 (1894), Contrib. U. S. Nat. Herb. 3:173. 1895.
V. paniculata × *stricta* Engelm. apud. Rydb. Contrib. U. S. Nat. Herb. 3:173. 1895.
V. bracteosa × *stricta* Rydb. Fl. Rock Mts. 740 (1917), Fl. Gen. Am. 678. 1932.
V. bracteosa × *stricta* Deam, in herb.
V. bracteosa × *stricta* Eggert, in herb.
V. stricta × *hastata* Eggert, in herb.

CHEROKEE Co., Cherokee, Oct. 17, 1924, Pammel (114,907); CLAY Co., Lake Twp., Sec. 26, pasture across the road north of Dewey's Pasture, July 23, 1935, Hayden 3,006; Meadow Twp., Sec. 32, wet land along Little Meadow Creek one mile north and two miles east of Spencer, Aug. 23, 1935, Hayden 3,003; DALLAS Co., Dawson, Aug. 16, 1918, Pammel (95,629); DECATUR Co., waste places, Aug. 25, 1904, Anderson (52,107); HANCOCK Co., Garner, July 27, 1918, Pammel (96,114); HARDIN Co., Eldora, Oct. 5, 1924, Pammel (116,221); LYON Co., Granite, Sept. 1, 1920, Pammel (97,-830); PALO ALTO Co., July 18, 1920, Pammel (97,793); Highland Twp., Sec. 30, moist ground between a moister strip occupied by *V. hastata* and a drier strip occupied by *V. stricta* in a pasture west of Virgin Lake, July 17, 1940, Hayden 7,370; STORY Co., Ames, 1902, Pammel (81,968); WINNESHIEK Co., Decorah, pasture in Upper Iowa River Valley, alluvial moist soil, July 23, 1934, Tolstead (144,284).

LABIATAE (Mint Family)

Perilla frutescens (L.) Britt. var. *crispa* (Benth.) Deane Purple Perilla
See Rhodora 25:40. 1923; and Flora of Indiana 826. 1940.

DAVIS Co., Salt Creek Twp., Sec. 2, about seven miles east of Floris at the edge of woods along the Des Moines River growing in sandy loam, Oct. 27, 1939, Hayden 9,630 and 9,690. This plant has been reported several times from the eastern part of the state and is known to grow along the Mississippi River in Illinois. It occurred near Floris in a patch about twelve feet in diameter and was growing luxuriantly. Though it is an annual, it has the reputation of spreading widely as an escape from cultivation. It is a native of the Himalayas, Burma, China, and Japan.

Physostegia virginiana (L.) Benth. var. *speciosa* (Sweet). Gray Syn. Flora N. A. Vol. II, Pt. I, 383. Showy False Dragonhead
Physostegia speciosa (Sweet) Sweet
Dracocephalum virginianum in part, of Britton & Brown
Physostegia virginiana in part, of Gray, Man. ed. 7 and *Dracocephalum virginianum* in part, of Britton and Brown, Illus. Flora, ed. 2.

ALLAMAKEE Co., Yellow River, Aug. 12, 1927, Pammel (129,913); CERRO GORDO Co., Mason City, Sept. 20-23, 1902, Pammel (26,188); CHICKASAW Co., 1925, Spiker (118,194 and 127,224); CLAY Co., Lake Twp., Sec. 34, semi-open, willow shaded bank along the Inlet of Round Lake, Aug. 10, 1934, Hayden 9,043; Meadow Twp., Sec. 29, in a hummock marsh in a cutoff of Meadow Creek, Aug .23, 1935, Hayden 9,044; CLAYTON Co., Guttenberg, Aug., 1876, Gmelin (94,820); McGregor, common in sandy, rocky soil, Aug. 12, 1925, Pammel (1,017); BUCHANAN Co., Hazelton, common in peaty marshes, Aug. 24, 1925, Pammel 764; BLACK HAWK Co., Cedar Falls, Aug. 13, 1908, Pammel (78,240); BOONE Co., Ledges, Aug., 1898, Pammel and Ball (12,180); DELAWARE Co., Aug., 1919, Bode (99,800); EMMET Co., low ground, river bank, Aug. 30, 1901, Cratty (19,366); near Mud Lake, Aug. 29, 1922, Wolden 677; FAYETTE Co., river banks, Fayette, July 30,

1894, Fink (83,634); FREMONT Co., wet soil, Aug. 8, 1902, Anderson (51,-722); HARRISON Co., Woodbine, Aug., 1925, Young (118,266); HARDIN Co., Steamboat Rock, Sept. 12, 1912, Pammel (52,649); HENRY Co., Mt. Pleasant, Mills (12,182); HUMBOLDT Co., Dakota City, Aug. 8, 1896, Pammel (12,181); JONES Co., Olin, July 28, 1903, Pike (28,395); LINN Co., Cedar Rapids, Aug., 1903, Buchanan (81,807); LUCAS Co., *Chariton*, Sept. 15, 1922, Pammel (106,736); LYON Co., Rock Rapids, Sept. 1, 1920, Pammel (97,846); MADISON Co., Sept. 7, 1919, Pammel (96,843); MARSHALL Co., Liscomb, July 26, 1913, Pammel (72,780); MITCHELL Co., Osage, 1914, Tuttle (74,175 and 73,671); MUSCATINE Co., Conesville, 1932, Melhus (140,023); STORY Co., Franklin Twp., Sec. 27, low prairie alluvial soil, Aug. 8, 1933, Hayden 436; TAMA Co., Tama, Clark Park, July, 1926, Fisk (126,782); WEBSTER Co., Ft. Dodge, 1932, Paige (130,811); WINNESHIEK Co., Decorah, July 21, 1881, Holway (38,923); WOODBURY Co., Sioux City Twp., growing in native garden at Morningside Branch Library near Morningside College, Sept. 10, 1938, Hayden (152,091); WORTH Co., peat bog near Fertile, Sept., 1908, Pammel (46,636).

This species was formerly mistaken for *P. parviflora* Nutt., which has petiolate leaves and smaller flowers.

SCROPHULARIACEAE (Figwort Family)

Verbascum phlomoides L.

JOHNSON Co., Iowa City, Sept., 1887, Hitchcock (18,992). This plant was first called to the attention of the writer by Dr. W. A. Anderson.

COMPOSITAE (Sunflower Family)

Antennaria fallax Greene

APPANOOSE Co., Udell Twp., about two miles north of Unionville on gravelly to sandy wooded ridges, April 22, 1938, Hayden 10,774; DAVIS Co., Lick Creek Twp., Sec. 26, one mile west of Floris on rocky gravelly eroded hills on the Hill Culture Experimental Farm, April 25, 1938, Hayden 10,775; WARREN Co., six miles east of Indianola in open woodland along South River near Highway 2, April 20, 1938, Hayden 10,773.

This species closely resembles *Antennaria plantaginifolia.*

Coreopsis tripteris L. var. *deamii* Standley. Rhodora 32:33. 1930.
Deams' Tall Tickseed

APPANOOSE Co., Bellaire Twp., Sec. 27, low, sandy soil two miles west of Centerville, Sept., 1940, Hayden 8,768; BOONE Co., the Ledges, Sept. 1, 1920, Cratty (96,823); DAVIS Co., Lick Creek Twp., Sec. 14, on roadside banks at the margin of woods one mile east of Floris, Oct. 3, 1938, Hayden 10,758; DECATUR Co., Anderson (1880); woods frequent, Aug. 15, 1897, Fitzpatrick (72,196); JASPER Co., Monroe, Sept. 29, 1929, Pammel (135,-060); LUCAS Co., Chariton, Sept. 15, 1922, Pammel (106,421); MADISON Co., Sept. 7, 1919, Pammel (96,906); MARSHALL Co., Liscomb, not uncommon, border of woods in Wisconsin drift sheet associated with *Monarda mollis, Astragalus canadensis, Solidago rigida, Aster laevis,* and *Lepachys*

pinnata. Aug., 1913, Pammel (73,325). POLK Co., Commerce, 1925, Pammel, Frankel and Riemen (1,288); STORY Co., Ames, high upland woods, Skunk River, Oct., 1912, Pammel (53,152); three miles northeast of Ames, Sept. 13, 1925, Cratty (117,692); VAN BUREN Co., Keosauqua, August, 1933, Fults (140,755); WAPELLO Co., Ottumwa, Sept. 6, 1930, Pammel (136,215); woodland banks in the vicinity of Cliffland near the Des Moines River, Oct. 3, 1938, Hayden 10,767.

This variety is represented in the herbarium of Iowa State College by fifteen of the seventeen sheets. It occurs in the southern half of the state.

Erigeron pulchellus Michx. Robin's Plantain

CLAYTON Co., Giard Twp., Sec. 14, four miles west of Marquette along Bloody Run Creek, June 16, 1940, Hayden 8,773; DELAWARE Co., Colesburg, May 28, 1939, Murley (734); DUBUQUE Co., Liberty Twp., Sec. 6, rocky soil in open woods along the Creek in Pine Hollow State Park two miles northwest of Luxemburg, June 17, 1940, Hayden 8,774; FAYETTE Co., Fayette, June, 1893, Fink (28,987); WINNESHIEK Co., Canoe Twp., Sec. 19, open woods on a north hillside, June 13, 1934, Tolsted (144,836); Decorah Twp., Sec. 6, May 20, 1934, Tolstead (144,384).

Haplopappus spinulosus (Pursh) DC subsp. *glaberrima* (Ryd.) Hall, Pub. 389 Carnegie Instit. Washington. 1928. Smooth Haplopappus
Sideranthus glaberrimus Ryd. Bull. Torr. Bot. Club 27:621. 1900.

CHEROKEE Co., Pilot Twp., Sec. 15, about three miles south of Cherokee on a hill north and opposite Pilot Rock, Sept. 5, 1937, Hayden 10,697; DICKINSON Co., Lakeville Twp., Sec. 21, dry, gravelly hills around a kettlehole on the Little Sioux River three miles west of Lake Okoboji, Aug. 23, 1938, Hayden 10,772; HARRISON Co., Missouri Valley, Aug. 9, 1894, Pammel 680; MILLS Co., Rawles Twp., Sec. 31, scattered on loess covered hills about three miles west of Tabor, Sept. 19, 1940, Hayden 8,807; POTTAWATTAMIE Co., prairie bluff, gravelly soil, Council Bluffs, July 14, 1926, Hayden 2,066; MONONA Co., on loess soil, Turin, Sept. 8, 1894, Pammel 684; SIOUX Co., Hawarden, Aug. 29, 1895, Pammel 681; WOODBURY Co., dry hills, Sioux City, Aug. 30, 1895, Pammel 682.

Eight of the thirteen sheets of the herbarium represent the smooth variety. Both the hairy and the smooth varieties are found chiefly in the counties bordering the Missouri River.

Lactuca campestris Greene. Pittonia 4:37-38. 1899. Field Wild Lettuce

ALLAMAKEE Co., Waterville, Aug. 23, 1920, Pammel (99,375); APPANOOSE Co., Centerville, Cooper Creek, Sept. 28, 1917, Pammel (73,259); BLACK HAWK Co., Island Y.M.C.A. Camp, July 9, 1925, Pammel (119,252); BOONE Co., Ogden, Aug. 20, 1898, Pammel (4,316); CHEROKEE Co., near Cherokee, Sept. 22, 1928, Pammel (132,715); CERRO GORDO Co., Mason

City, Aug. 12, 1922, Pammel (106,229); CRAWFORD Co., Denison, Aug. 15, 1927, Blume (129,620); CLAY Co., Lake Twp., Sec. 25, common on open roadside banks, July 9, 1940, Hayden 9,894; DICKINSON Co., dry prairies July 23, 1931, Cratty (97,689); EMMET Co., Armstrong, Paige (130,742); GREENE Co., four miles north of Scranton, Sept. 18, 1920, Cratty (98,782); HENRY Co., near Salem, Sept. 30, 1917, Pammel and Jaques (73,369); MARSHALL Co., Marshalltown, Sept. 13, 1902, Pammel (85,903); PALO ALTO Co., Lost Island Twp., Sec. 30, open roadside about five miles northeast of Lost Island Lake, July 8, 1940, Hayden 9,893; POLK Co., Des Moines, Sept. 16, 1916, McKune (70,773).

Silphium integrifolium Michx. var. *deamii* Perry. Rhodora 39:287. 1937.
Deam's Rosin-weed

CLINTON Co., Clinton, Aug. 16, 1907, Pammel (107,571); DALLAS Co., Adel, Aug. 21, 1907, Clark (108,115).

Solidago altissima L. Sp. Pl. 878. 1753. Tall Goldenrod

ALLAMAKEE Co., Waukon, Sept. 2, 1919, Pammel (96,299); CERRO GORDO Co., Mason City, common, border of peat bog between two limestone ledges, Sept. 1, 1925, Pammel, Naylor and McNider (119,408); Clear Lake, Sept. 12, 1924, Pammel (114,380); CLAY Co., Freeman Twp., Sec. 17, grassy bank bordering Pickerel Run at the entrance to Dickens, Aug. 7, 1934, Hayden 10,670; Lake Twp., Sec. 25, associated with *S. altissima* var. *hargeri* in Dewey's Pasture eight miles north of Ruthven, Aug. 24, 1935, Hayden 10,676; Peterson Twp., Sec. 34, low prairie one mile east of Peterson, Sept. 5, 1936, Hayden 10,672; CLAYTON Co., McGregor, Sept., 1919, Hayden (97,203); Strawberry Point, Sept., 1922, Pammel (106,628); DAVIS Co., Prairie Twp., Sec. 16, prairie one mile east of Pulaski, Sept. 24, 1940, Hayden 8,834; FAYETTE Co., Fayette, Aug., 1893, Fink (26,550); FLOYD Co., Floyd Springs, Tuttle, Sept. 20, 1918; HAMILTON Co., Cass Twp., Sec. 29, prairie along the Chicago and Northwestern railroad track two miles north of Webster City, Aug. 21, 1931, Hayden 10,683; PALO ALTO Co., Highland Twp., Sec. 6, roadside one mile east of Ruthven along Highway 341, Sept. 1, 1934, Hayden 10,671; Lost Island Twp., Sec. 30, depression along roadside east of Lost Island Lake, Oct. 10, 1936, Hayden 10,673; Highland Twp., Sec. 30, two miles south of Ruthven prairie southwest of Virgin Lake, Sept. 12, 1936, Hayden 10,680; POLK Co., Saylor Twp., Sec. 13, along the edge of a marsh at the intersection of Highways 6 and 169 on Fourteenth Street in Des Moines, Sept. 6, 1937, Hayden 10,679; STORY Co., Ames, roadsides, Aug., 1883 Hitchcock (14,404).

Solidago altissima L. var. *hargeria* (Fernald) Shinners
See Rhodora 17:11. 1915; also Unpub. Man., Shinners
S. canadensis L. var. *gilvocanescens* of manuals.

ALLAMAKEE Co., Yellow River near Waukon, Aug. 22, 1920, Pammel (97,454); BOONE Co., Worth Twp., Sec. 28, three miles west of Luther in

a springy shaded area of a tributary of the Des Moines River, Oct. 22, 1938, Hayden 10,688; BLACK HAWK Co., dry roadside on Highway 10, ten miles west of Oelwein, Aug. 20, 1933, Hayden 344; CERRO GORDO Co., Clear Lake, common at border of woods in Clear Lake State Park, Sept. 1, 1925, Pammel 526; CHICKASAW Co., New Hampton, common along fencerows and roadsides, Aug. 21, 1925, Pammel and Spiker (686); CLAY Co., Lake Twp., Sec. 35, in open ground among trees west of Trumbull Lake, Aug. 21, 1936, Hayden 10,675; Freeman Twp., Sec. 3, south bank of Round Lake, Sept. 23, 1931, Hayden 10,677; CLAYTON Co., McGregor, Sept., 1919, Hayden 97,194; Clayton, Aug. 4, 1923, Pammel (110,510); CLINTON Co., Clinton, Sept. 10, 1924, Pammel (114,411); DAVIS Co., Lick Creek Twp., Sec. 26, at edge of woods, along Lick Creek on the Hill Culture Experimental Farm one mile west of Floris, Oct. 7, 1939, Hayden 9,672; DALLAS Co.. Dawson, Aug. 16, 1918, Pammel (95,636); EMMET Co., Armstrong, Aug. 21, 1917, Cratty (14,394); FREMONT Co., Hamburg, Sept. 19, 1920, Pammel (98,344); FLOYD Co., Charles City, Sept., 1919, Pammel (93,931); GRUNDY Co., Grundy Center, prairies, Aug. 23, 1925, Pammel 248; HAMILTON Co., Cass Twp., Sec. 29, two miles north of Webster City in prairie bordering woodland, Aug., 1933, Hayden 475; HARDIN Co., Radcliffe, common in prairie roadsides border of woods and fields, Sept. 5, 1925, Pammel 804; HENRY Co.. near Salem, Sept. 30, 1917, Pammel 183; HOWARD Co., Saratoga, Sept. 2, 1919, Pammel (96,281); JASPER Co., Split Rock, Aug. 3, 1922, Hansen (108,390); JOHNSON Co., Iowa City, Hitchcock (1,440); LINN Co., Cedar Rapids, Sept. 12, 1891, Pammel (14,400); LYON Co., Rock Rapids, Aug. 29, 1918, Pammel (95,501); MARSHALL Co., Marshalltown, Sept. 12, 1891, Stewart (14,413); PALO ALTO Co., Highland Twp., Sec. 6, growing in colonies in openings of the sandy wooded south shore of Lost Island Lake, Aug. 25, 1937, Hayden 10,669; one mile east of Lost Island Lake, Lost Island Twp., Sec. 29, open grassland on slopes around Johnson's slough, Aug. 14, 1939, Hayden 9,781; POLK Co., Des Moines. Sept. 26, 1902, Pammel (33,344); TAMA Co., Traer, Oct., 1922, Pammel and Becraft (103,918); STORY Co., Ames, Sept. 20, 1874, Wattles (14,392); Sept. 15, 1816, Bessey (14,395); UNION Co., Creston, Andrews (22,806); VAN BUREN Co., Keosauqua, a common weed in alfalfa fields, Sept. 9, 1925, Pammel 789; WINNEBAGO Co., Lake Mills, Sept., 1908, Pammel (70,657); WINNESHIEK Co., near Bluffton, prairie roadside, July 18, 1933, Tolstead (144,361).

S. canadensis apparently does not occur as far west as Iowa. The above variety is the prevailing one.

Solidago graminifolia (L.) Salisb. var. *remota* (Greene) Harris. Rhodora 45:413. 1943. Solitary-headed Bushy Goldenrod
Euthamia remota Greene
Solidago remota (Greene) Friesner

PALO ALTO Co., Emmetsburg Twp., Sec.15, sandy roadside and pasture nine miles east of Ruthven, Sept. 29, 1935, Hayden 10,644.

Solidago gymnospermoides (Greene) Fernald Viscid Bushy Goldenrod
Euthamia gymnospermoides Greene

DECATUR Co., Sept. 19, 1904, Anderson (51,678); EMMET Co., Estherville Twp., Sec. 14, in an old gravel pit along the railroad tracks on the south side of Estherville, Sept. 1, 1941, Hayden 8,825; PALO ALTO Co., Highland Twp., Sec. 15, three miles east of Lost Island Lake and one mile north of Highway 18, gravelly bank by roadside, Sept. 16, 1938, Hayden 10,685; Highland Twp., dry, sandy field four miles east of Ruthven, Weber and Hayden 1,167; WAYNE Co., Benton Twp., Sec. 24, bordering a small pond west of Corydon, Sept. 26, 1940, Hayden 8,826.

Solidago pruinosa Greene. Pittonia 4: 70. 1899. Hoary Goldenrod
See Contr. Nat. Herb. 3: 162. 1895.
S. canadensis L. var. *gilvocanescens* Rydb.

BOONE Co., Luther, Aug. 24, 1929, Pammel (133,970); CHEROKEE Co., Cherokee, Sept. 5, 1920, Pammel (97,986); CLAY Co., Gillett Grove, Sept. 28, 1912, Pammel (53,486); Sioux Twp., Sec. 16, two miles east of Spencer by the Little Sioux River, July 15, 1939, Hayden 9,799; CLARKE Co., eight miles north of Osceola, Sept. 26, 1924, Pammel and Pammel (114,341); EMMET Co., Emmet Twp., prairie, Aug. 4, 1922, Wolden 594; High Lake, Sept. 14, 1922, Wolden 708; FREMONT Co., Sidney, Aug. 25, 1928, Pammel and Johnson (135,030); HENRY Co., Mt. Pleasant, 1918, Jaques (94,999); JASPER Co., Newton, Sept. 15, 1891, Drew (14,390); KOSSUTH Co., Algona, Sept. 19, 1926, Pammel and Halloway (126,258); LUCAS Co., Little Rock, Aug. 29, 1918, Pammel (95,522); MARSHALL Co., Marshalltown, Sept. 12, 1891, Stewart (14,414); MONONA Co., Turin, Sept. 8, 1894, Pammel (14,410); POLK Co., Des Moines, July 14, 1897, Pammel (14,415); SCOTT Co., 1897, LeBuhn (14,393); SIOUX Co., Hawarden, Aug., 1895, (14,403); STORY Co., Ames, Sept. 20, 1889, Schulte (14,412); VAN BUREN Co., Keosauqua, abundant in more or less weedy fields with *Ambrosia artemisiaefolia, Nepeta cateria, Bidens vulgata,* and *Panicum dichotomiflorum,* Sept. 10, 1925, Pammel and Reis, 472; WEBSTER Co., Dayton, common on Wisconsin Drift Sheet, somewhat weedy, associated with *Helianthus grosseserratus, H. annuus, Iva xanthifolia,* and *Aster multiflorus,* Aug. 21, 1926, Pammel 12; WINNESHIEK Co., northeastern Iowa, Aug. 14, 1895, Goddard (77,914); WOODBURY Co., Sioux City, woods between mounds, Aug. 30, 1895, Pammel (14,401); WRIGHT Co., Eagle Grove, Aug. 21, 1891, Pammel (14,411); Belmond, Sept., 1908, Pammel (83,231).

This goldenrod is not described in the manuals. It was called to the attention of the writer by Dr. L. H. Shinners and is discussed by him in unpublished studies of midwestern goldenrods. This species is the earliest blooming of the *S. canadensis gilvocanescens—S. altissima* complex which it precedes by nearly a month, at least in northern Iowa, where it flowers from July 20 to about Aug. 20. In a botanical survey of Clay and Palo Alto Counties, Iowa (Hayden, 1943), its flowering period was not distinguished from that of *S. altissima* var. *hargeri* there referred to as *S. canadensis gilvocanescens* whose blooming time it partly overlaps.

Tragopogon dubius Scop.

See Illust. Flora von Mittel-Europa VI. 1046.

ADAIR Co., July 15, 1930, Driscoll (136,637); BENTON Co., Norway, 1929, Springer (134,409); CARROLL Co., Carroll, July, 1923, Co. Agt. (109,729); CASS Co., Massena, June 29, 1923, Curley (109,771); CRAWFORD Co., Aug., 1928, Butler (133,072); DALLAS Co., Woodward, July 10, 1925, Miller (134,314); FREMONT Co., Sidney, July 6, 1920, Hiezer (97,768); GUTHRIE Co., Jamaica, July, 1918, Thompson (84,218); MONONA Co., May 25, 1924, Pine (133,740); PAGE Co., Northboro, June 11, 1927, Boylan (129,772); RINGGOLD Co., Mt. Ayr., July 1, 1922, Blair (102,992); SAC Co., Lake View, July 19, 1924, Pammel (115,110); SIOUX Co., Hull, June 20, 1929, Reylts (134,451); STORY Co., July 20, 1928, Pammel and Johnson (132,813); TAYLOR Co., Bedford, June, 1929, Isaacs (134,411); WINNESHIEK Co., two miles west of Ossian growing along roadside, July 3, 1933, Tolstead (144,357).

The eight to nine involucral bracts, equal to or shorter than the chrome-yellow corollas, distinguishes *T. pratensis* from *T. dubius* which has ten to thirteen involucral bracts much longer than the lemon-yellow corollas. The latter usually has peduncles fistulose at the top. Both are adventive from Europe. According to Kearney and Peebles (1942) the range of *T. dubius* is Colorado to Idaho, New Mexico, and Arizona. It has also been reported from Indiana by Hull (1943), and by Gray Herbarium from Virginia, Michigan, Illinois, Minnesota, South Dakota, Oklahoma, Texas, Washington, Oregon, and California.

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FLORA OF ALASKA AND ADJACENT PARTS OF CANADA[1]

An Illustrated and Descriptive Text of All Vascular Plants Known to Occur Within the Region Covered

PART III. *CYPERACEAE TO ORCHIDACEAE*

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From the Department of Botany, Iowa State College

Received June 20, 1944

7. CYPERACEAE (Sedge Family)

Grass-like or rush-like plants with usually solid stems and three-ranked leaves with closed sheaths and narrow blades. Flowers in spikes or spikelets, in the axil of two-ranked or spirally arranged scales; perianth composed of bristles, a sac-like organ (perigynium), or wanting; stamens and styles two or three; anthers two-celled, basifixed; ovary one-celled, one-ovuled; fruit an achene.

1A. Fertile flowers all perfect, sometimes staminate flowers present.
 1B. Base of style persistent as a tubercle.
 1C. Basal empty scales several.....1. *Rynchospora*
 2C. Basal empty scales not more than two or three..2. *Eleocharis*
 2B. Base of style not persistent.
 1C. Bristles six to many, much elongated.....3. *Eriophorum*
 2C. Bristles few, short 4. *Scirpus*
2A. All flowers unisexual.
 1B. Pistillate flower partly enwrapped in the scale....5. *Kobresia*
 2B. Pistillate flowers enclosed in a sac 6. *Carex*

1. RYNCHOSPORA Vahl.

Leafy perennials with erect stems; leaves narrow, flat or involute; spikelets clustered, ovoid, oblong, or fusiform; scales imbricate, thin, one-nerved, usually mucronate by the excurrent midvein; perianth of one to twenty-four, mostly six, barbed or scabrous bristles; achenes lenticular, capped by the persistent base of the style or the whole style. (Greek, referring to the beak-like tubercle.)

R. alba (L.) Vahl. White Beaked-rush

Stem slender, glabrous, 10–25 cm. tall; leaves bristle-like, 1 mm. or less wide; spikelets several or numerous, 4–6 mm. long, in one to four dense heads; scales light-colored, acute; bristles nine to fifteen, downwardly barbed, about as long as the achene and the tubercle.

Southeastern Alaska; has an interrupted circumboreal distribution. (Fig. 192.)

[1] Preceding parts of this paper were published in this Journal as follows: Part 1, Vol. XVIII, pp. 137–175, 1943; Part 2, Vol. XVIII, pp. 381–446, 1944.

2. ELEOCHARIS R. Br.

Rush-like tufted plants growing in water or wet places; leaves reduced to mere sheaths or rarely the lower blade-bearing; inflorescence an erect, terminal spikelet; perianth of one to twelve, usually retrosely barbed, bristles; base of styles persistent, forming a tubercle at the summit of the achene. (Greek, referring to the growth of most species in marshy ground.)

1A. Culms low, 3–15 cm. long.
 1B. Achene reticulate 1. *E. acicularis*
 2B. Achene rough 2. *E. nitida*
2A. Culms taller, 12–150 cm. long.
 1B. Achenes finely papillose 3. *E. kamtschatica*
 2B. Achenes smooth.
 1C. Tubercle more than one-half as wide as the achene..... 4. *E. uniglumis*
 2C. Tubercle less than one-half as wide as the achene.
 1D. Tubercle conical-triangular 5. *E. palustris*
 2D. Tubercle cap-like 6. *E. mamillata*

1. *E. acicularis* (L.) R. & S. Needle Spike-rush
Scirpus acicularis L.

Stems filiform, grooved, obscurely four-angled, 3–10 cm. tall; spikelet 3–6 mm. long; three- to ten-flowered; scales oblong, pale green, usually with a brown band on each side of the midvein; bristles three or four, short and fugacious; achenes pale, obscurely three-angled, with intermediate ribs; tubercle conic, about one-fourth as long as the achene.

From Seward Peninsula south and east. Circumboreal. (Fig. 193.)

2. *E. nitida* Fern. Slender Spike-rush
E. tenuis (Willd.) Schult.
Scirpus nitidus (Fern.) Hult.

Perennial by slender rootstocks; culms slender, tufted, four-angled, striate, 2–8 cm. tall; tips of upper sheaths whitish; spikelets 2.5–4.5 mm. long, 1.5–2.5 mm. wide; scales ovate or ovate-oblong, the tips obtuse, purplish-brown with greenish midrib and narrow, scarious margins; bristles two to four, shorter than the achene, fugacious, or wanting; achene trigonous, very minutely roughened, 1 mm. or less long, tubercle conic, short, acute.

Western Pacific district and Ottawa Valley—Newf.—N. S.—N. Hamp.

3. *E. kamtschatica* (C. A. Mey.) Kom. Kamchatka Spike-rush
Scirpus kamtschaticus C. A. Mey.

Stems erect, 1–4 dm. tall; spikelet ovoid, 6–15 mm. long; scales ovate, purplish-brown with reddish midvein; bristles about as long as the achene and tubercle; achene greenish-yellow, about 1.5 mm. long, finely papillose; tubercle nearly as large as the achene, cap-like.

Eastern Asia and the Bering Sea and Aleutian regions—Southeastern Alaska. (Fig. 194.)

4. *E. uniglumis* (Link) Schult. One-bracted Spike-rush
Scirpus uniglumis Link.

Stoloniferous and loosely caespitose; culms slender, 5–70 cm. tall, reddish at the base; spikelet 5–15 mm. long, 2–6 mm. thick, five- to thirty-flowered; basal scale roundish, completely clasping the base of the spikelet; fertile scales castaneous or purplish, firm, lustrous, 3–5 mm. long; achenes obovoid, yellowish or darker, tubercle conic-ovoid, one-half to two-thirds as wide as the achene.

Collected at Circle and at Hyder, a variable, circumboreal species. (Fig. 195.)

5. *E. palustris* (L.) R. & S. Creeping Spike-rush
Scirpus palustris L.

Stems erect, striate, 3–15 dm. tall; spikelet ovoid-cylindric, 8–20 mm. long, many-flowered; scales brown with scarious margins; bristles usually four, longer than the achene and tubercle; achene lenticular, smooth, yellowish; tubercle conic-triangular, flattened, 0.25–0.5 as long as the achene.

Central Alaska south and east; circumboreal. (Fig. 196.)

6. *E. mamillata* Lindb. f. Pale Spike-rush
Scirpus mamillatus Lindb. f.

Resembling *E. palustris;* culms 2–12 dm. tall, pale; spikelet subcylindric to lanceolate, 1–3 cm. long, 2–5 mm. thick, many-flowered, acute, scales narrowly ovate, obtuse to subacute, appressed, 2–4 mm. long; achenes yellow or pale brown; tubercle yellow, small, cap-like.

Pacific Coast districts; circumboreal.

3. ERIOPHORUM L.

Bog plants with erect stems and linear leaves or the upper one or two reduced to bladeless sheaths; spikes terminal, solitary and capitate, or several in an involucrate umbel; scales spirally imbricated; flowers perfect; perianth of soft capillary bristles which are much exerted at maturity; achenes three-angled, oblong, ellipsoid, or obovoid. (Greek, wool-bearing.)

1A. Spike solitary.
1B. Bristles six 1. *E. alpinum*
2B. Bristles numerous.
1C. Plants stoloniferous.
1D. Anthers 0.5–1 mm. long, bristles white 2. *E. scheuchzeri*
2D. Anthers longer.
1E. Middle scales blunt with broad hyaline margins 3. *E. chamissonis*
2E. Middle scales acute with narrow hyaline margins 4. *E. medium*

2C. Plants densely tufted, no stolons.
3D. Scales gray, translucent 5. *E. vaginatum*
4D. Scales grayish or greenish-black, not translucent.
1E. Plant 6–20 cm. tall 6. *E. callitrix*
2E. Plants 3–7 dm. tall 7. *E. brachyantherum*
2A. Spikes more than one.
1B. Leaf-blades triangular-channeled throughout
..... 8. *E. gracile*
2B. Leaf-blades flat below the middle.
1C. Midrib of the scales prominent to the very tip
..... 9. *E. viridi-carinatum*
2C. Midrib of scales not prominent at the tip
.....10. *E. angustifolium*

1. *E. alpinum* L. Alpine Cotton-grass

Stems scattered or somewhat tufted, triangular, 10–25 cm. tall; leaves subulate, 6–20 mm. long, borne near the base, lower sheaths often bladeless; involucral bract blunt-subulate, shorter than the spike; spike small, erect; glumes yellowish-brown with slender midvein; bristles six, white, flat, crisped, 10–20 mm. long; achene obovate, apiculate.

Cook Inlet—central Alaska—Hudson Bay—Conn.—Mich.—B. C. (Fig. 197.)

2. *E. scheuchzeri* Hoppe. White Cotton-grass

Stems slender, 2–6 dm. tall; sheaths all blade-bearing except the uppermost one; blades filiform, channeled, shorter than the culm; spike erect, globose at maturity; bristles numerous, white, or in drying often yellowish, 15–30 mm. long; achenes narrowly oblong, acute with a subulate beak, scarcely 2 mm. long.

Throughout our area; circumboreal. (Fig. 198.)

3. *E. chamissonis* C. A. Mey. Russet Cotton-grass
E. russeolum Fries.

Culms triangular, 3–7 dm. tall; upper sheaths somewhat inflated. This species closely resembles *E. scheuchzeri* but is of taller growth, the scales are broader with wide, hyaline margins, the achene is broader and narrowed at the base, the numerous bristles 2–4 cm. long and usually of a russet-brown color, although a pale form occurs. This pale form which is usually nearly white is the only one found in the Bering Sea region and on the Arctic Coast. It has been described as var. *albidum* Fern. (var. *leucothrix* (Blomg.) Hult.

Throughout our area; circumboreal. (Fig. 199.)

4. *E. medium* Anders.

This name is applied to plants forming a connecting link between *E. chamissonis* and *E. scheuchzeri*. It is probably a hybrid of these two species and occurs where both parent species are found, but according to Hultén it does not occur in regions where *E. scheuchzeri* alone is found. In

E. scheuchzeri the anthers are 1 mm. or less in length, in *E. chamissonis* they are 2–3 mm. long, and in *E. medium* they are 1-2 mm. long. The bristles are tinged with brown.

5. *E. vaginatum* L. Niggerheads, Sheathed Cotton-grass

Densely tufted, forming "niggerheads"; culms stiff, obtusely triangular, 2–5 dm. tall; leaves filiform, triangular, channeled; upper sheaths inflated; spike oblong, 1–3 cm. long; glumes ovate-lanceolate, acuminate, thin, mostly hyaline; anthers 2–3 mm. long; bristles white or slightly dingy, 10–16 mm. long; achene narrowly ovoid, scarcely apiculate. Ssp. *spissum* (Fern.) Hult. (*E. spissum* Fern.) spikes ovoid to subglobose, the rachis 6–10 mm. long compared to 9–15 in the type form, anthers 1–2 mm. long, achenes broadly ovoid. This is the form found in the Bering Sea and Arctic regions.

The typical form from central Alaska eastward; circumboreal. (Fig. 200.)

6. *E. callitrix* Cham. Arctic Cotton-grass

Culms low, 6–20 cm. tall; usually only one sheath which is close to the base and often bears a short blade; leaves rigid, spreading, the blades forming an angle with the sheath; scales nearly uniform in color; bristles pure white.

Northeastern Asia and Bering Sea islands—Baffin Island—E. Greenl.—N. Newf.

7. *E. brachyantherum* Trautv. Close-sheathed Cotton-grass
E. opacum Am. Auct.

Culms 3–7 dm. tall, from dense tussocks; basal leaves elongate, continuous with the sheath; uppermost sheath scarcely inflated; scales dark, ovate-lanceolate or the inner linear-lanceolate, acuminate; bristles white or slightly tinged brown, 1–2 cm. long; achenes obovate-oblong, smooth, conspicuously apiculate.

Throughout most of our territory; circumboreal. (Fig. 201.)

8. *E. gracile* Koch. Slender Cotton-grass

Culms slender, smooth, terete, 3–6 dm. tall; sheaths all blade-bearing, the blades narrowly linear, not over 2 mm. wide; spikes two to six, some of them on slender, drooping, pubescent peduncles; scales ovate with prominent midribs; bristles white, 15–25 mm. long; achenes linear-oblong, about 2.5 mm. long.

Central Alaska east and south; circumboreal. (Fig. 202.)

9. *E. viridi-carinatum* (Engelm.) Fern. Thin-leaved Cotton-grass

Similar in appearance to *E. angustifolium;* leaves thin, flat, black at the base; spikes usually numerous, up to thirty; peduncles finely hairy, elongated or short; scales ovate-lanceolate, the midvein extending to the tip and sometimes excurrent; achene oblong-ovoid; bristles white or slightly yellowish.

Sphagnum bogs, Cook Inlet region and B. C.—Hudson Bay—Newf.—N. Y.—Ohio—Wyo.

10. *E. angustifolium* Roth. Tall Cotton-grass

Culms smooth, obtusely triangular above 3–7 dm. tall; leaf-blades more than 3 mm. wide; bracts two to four, often blackish at the base; spikes two to twelve, in a terminal umbel; peduncles smooth; scales ovate-lanceolate, purple-green or brown; bristles white or tawny, up to 3 cm. long; achenes nearly black, sharp-pointed, about 2.5 mm. long. Forms of this species have been reported as *E. polystachyon* L.

Common throughout our territory; circumboreal. (Fig. 203.)

4. SCIRPUS L.

Ours all perennials of swamps or wet places; leaves grass-like or in some species reduced to sheaths; spikelets solitary, clustered, or umbellate, the inflorescence usually subtended by one or more leafy bracts, often appearing lateral; scales arranged spirally, the lower often empty; flowers perfect; perianth of one to six usually barbed or pubescent bristles; styles and stamens two or three. (Latin name for the bulrush.)

1A. Spikelet small, solitary, terminal.
 1B. None of the sheaths leaf-bearing 1. *S. pauciflorus*
 2B. One or more of the sheaths leaf-bearing 2. *S. caespitosus*
2A. Spikelets normally more than one.
 1B. Spikelets few, appearing lateral 3. *S. americanus*
 2B. Spikelets several.
 1C. Spikelets spicate 4. *S. rufus*
 2C. Spikelets umbellate 5. *S. pacificus*
 3B. Spikelets numerous.
 1C. Sheaths bladeless, culms terete 6. *S. validus*
 2C. Plant leafy, culms triangular 7. *S. microcarpus*

1. *S. paucifloris* Light. Few-flowered Club-rush

Similar in appearance to the common *C. caespitosus* but less densely tufted; culms three-angled; upper sheath truncate, without trace of a leaf; no involucral bract; bristles two to six, hispid.

Known from Manly Hot Springs and B. C.—Que.—N. Y.—Calif.

2. *S. caespitosus* L. var. *callosus* Bigel. Tufted Club-rush
S. caespitosus L. ssp. *austriacus* (Pella) Achers. & Graebn.

Culms slender, densely tufted, 1–3 dm. tall; basal sheaths numerous, the upper one bearing a short blade; spikelet 4–5 mm. long, glumes yellowish-brown; bristles six, smooth, longer than the acute achene.

Central Alaska southward; circumboreal. (Fig. 204.)

3. *S. americanus* Pers. Three-square

Culms sharply triangular, erect, 3–12 dm. tall; leaves 1–3, linear, keeled, shorter than the culm; spikelets one to seven, oblong-ovoid, acute, 8–15 mm. long, appearing as if lateral; bract 2–10 cm. long; glumes broadly

ovate, brown, often emarginate or two-cleft; awned; bristles two to six, barbed.

Circle Hot Springs and B. C.—Newf.—Bermuda—S. Am.—Calif.—Europe. (Fig. 205.)

4. *S. pacificus* Britt. Pacific Bulrush

Culms leafy, stout, sharply three-angled with flat sides, 5–8 dm. tall; leaves 1 cm. or less wide, the longer often as long as the culm; bracts two to five, some of them longer than the inflorescence; spikelets ovoid, 1–2 cm. long, usually densely clustered; scales brown-tipped with a recurved awn; bristles shorter than the achene; achene light brown, about 2.5 mm. wide, nearly 4 mm. long.

Saline marshes along the coast, Anchorage—s. Calif. (Fig. 206.)

5. *S. rufus* (Huds.) Schrad. Red Club-Rush

Culms in small clusters from slender rootstocks, erect, 8–30 cm. tall; leaves narrow, channeled, up to 15 cm. long, the lower reduced; spikelets reddish-brown, few-flowered, ovoid-oblong, 5–7 mm. long, in a terminal two-ranked spike 1–2 cm. long; bract 5–25 mm. long; scales lanceolate, acute, one-nerved; bristles one to six, shorter than the achene, deciduous.

Matanuska—N. W. Terr.—Newf.—N. S.—James Bay. Also N. Europe. (Fig. 207.)

6. *S. validus* Vahl. Great Bulrush

S. lacustris Am. Auct.

Culms stout, terete, smooth, spongy, 1–3 m. tall, 1–2 cm. thick, sheathed below; spikelets 5–12 mm. long, numerous in a compound cluster; scales ovate to suborbicular, reddish-brown, with strong midrib; achenes gray, plano-convex, about 1.5 mm. by 2 mm., bristles four to six, downwardly barbed.

Cook Inlet region—Newf.—West Indies—Calif. (Fig. 208.)

7. *S. microcarpus* Presl. Small-fruited Bulrush

Culms stout and leafy, 6–15 dm. tall; leaves 7–18 mm. wide, up to 1 m. long, rough-margined; spikelets very numerous in a very compound inflorescence, ovoid-oblong, acute, 3–4 mm. long; scales greenish; bristles four, barbed, longer than the smooth whitish achene.

Western Pacific Coast of Alaska—Newf.—Conn.—Calif. (Fig. 209.)

5. KOBRESIA Willd.

Slender arctic and mountain sedges; culms erect, leafy below; spikelets very small, one- or two-flowered, in our species arranged in spikes; stamens three; perianth bristles and perigynium wanting; ovary oblong, narrowed into the style; stigmas two or three, linear; achenes obtusely three-angled, sessile. (von Kobres was a naturalist of Augsberg, Germany.)

Spike one 1. *K. myosuroides*
Spikes more than one 2. *K. simpliciuscula*

1. *K. myosuroides* (Vill.) Fiori & Paol. Bellard Kobresia
K. bellardii (All.) Degland.

Culms tufted, very slender, 1–4 dm. tall, longer than the leaves; leaves near the base, 2–20 cm. long, 0.25–0.5 mm. wide, acicular; spike bractless, 1–3 cm. long, 2–4 mm. in diameter, the terminal spikelet staminate, the lateral ones with one staminate and one pistillate flower; scales 2–3 mm. long; achenes about 2.5 mm. long, 1 mm. wide.

Arctic and alpine; circumpolar. (Fig. 210.)

2. *K. simpliciuscula* (Wahl.) Mack.

Culms and leaves similar to *K. myosuroides;* spikes three to ten, 3–8 mm. long, 1.5–2.5 mm. wide, in a head 10–35 mm. long, which sometimes appears spike-like; terminal spikelets staminate, the lateral androgynous or pistillate and one-flowered; achenes about 3 mm. long, 0.5 mm. wide.

Bering Sea and Alaska Range regions; circumpolar.

6. CAREX L.

Perennial grass-like sedges with mostly triangular stems (culms) and three-ranked leaves, the upper (bracts) subtending the spikes or wanting; plants monoecious or sometimes dioecious; spikes one-many, either wholly staminate, wholly pistillate, or producing both staminate and pistillate flowers in different ends of the same spike; flowers solitary in the axils of scales; perianth none; staminate flowers of three (rarely two) stamens with filiform filaments; pistillate flowers of a single pistil with a style and two or three stigmas, forming an achene enclosed in a sac (perigynium) through the orifice of which the stigmas protrude; achenes triangular, lenticular, or plano-convex and enclosed in the perigynium or rarely rupturing it. (Greek, to cut, on account of the sharp leaves.)

A vast genus, well represented in our area. The division of genus here adopted is that of Kukenthal which is much easier to use though more artificial than that adopted for the American species by Mackenzie. The illustrations are of glume, perigynium and achene. They are not drawn to any particular scale but the parts illustrated are in proportion for that species.

1A. Spike single, terminal *Primocarex*
2A. Spikes two or more.
1B. Spikes sessile, bisexual *Vignea*
2B. Spikes peduncled, usually unisexual, sometimes bisexual.
1C. Stigmas two *Eucarices distigmaticae*
2C. Stigmas three *Eucarices tristigmaticae*

Primocarex

1A. Pistillate scales persistent.
1B. Stigmas two.
1C. Spike androgynous.
1D. Perigynia with rounded base 3. *C. capitata*

2D. Perigynia tapering to a stipulate base.
1E. Beak scabrous, leaves filiform 1. *C. nardina*
2E. Beak smooth, leaves flat 2. *C. jacobi-peteri*
2C. Spike unisexual 4. *C. gynocrates*
2B. Stigmas three.
1C. Perigynia lanceolate with long beak.
1D. Leaves flat, rhizomes long 6. *C. anthoxanthea*
2D. Leaves canaliculate, rhizomes short..... 7. *C. circinata*
2C. Perigynia with short beak or beakless.
1D. Spike unisexual 5. *C. scirpoidea*
2D. Spike androgynous.
1E. Perigynia beakless, flat 8. *C. leptalea*
2E. Perigynia with short beaks, trigonous.
1F. Perigynia obovate.
1G. Leaves filiform, plant tufted 9. *C. filifolia*
2G. Leaves keeled or flat, plant with creeping rhizomes 10. *C. rupestris*
2F. Perigynia ovate 11. *C. obtusata*
2A. Pistillate scales early deciduous.
1B. Spike densely flowered, only lower perigynia reflexed.
1C. Stigmas two 12. *C. pyrenaica*
2C. Stigmas three 13. *C. nigricans*
2B. Spike few-flowered, perigynia all reflexed in age.
1C. Perigynia 6–7 mm. long 14. *C. pauciflora*
2C. Perigynia 4–5 mm. long 15. *C. microglochin*

Vignea

1A. Spikes androgynous.
1B. Stigmas two.
1C. Rhizome long, creeping.
1D. Perigynia not wing-margined.
1E. Spikes densely aggregated, perigynia inflated 16. *C. maritima*
2E. Spikes distinct, perigynia not inflated.
1F. Rootstock slender, leaves 1–1.5 mm. wide 17. *C. stenophylla*
2F. Rootstock stout, leaves 1.5–3 mm. wide 19. *C. praegracilis*
2D. Perigynia wing-margined 18. *C. chordorrhiza*
2C. Rhizome short, plants tufted.
1D. Leaves 4–8 mm. wide 20. *C. stipata*
2D. Leaves 1–2.5 mm. wide 21. *C. diandra*
2B. Stigmas three 22. *C. macrocephala*
2A. Spikes gynaecandrous.
1B. Margins of perigynia winged.
1C. Spikes aggregated into a dense head.
1D. Bracts leaf-like, exceeding the head....23. *C. athrostachya*

2D. Bracts shorter than the head.
1E. Perigynia very conspicuous 24. *C. macloviana*
2E. Perigynia not conspicuous 26. *C. phaeocephala*
2C. Spikes not aggregated into a head.
1D. Perigynia lanceolate 27. *C. crawfordii*
2D. Perigynia ovate.
1E. Beak of perigynia flattened and serrulate to the tip 28. *C. aenea*
2E. Beak of perigynia terete, not serrulate toward the tip 25. *C. praticola*
2B. Margins of the perigynia not winged.
1C. Perigynia white-puncticulate, beak short.
1D. Plants tufted, lacking stolons.
1E. Spikes two to four, congested.
1F. Leaves 2 mm. wide, flat 29. *C. lachenalii*
2F. Leaves narrower.
1G. Culms scabrous, scales yellowish-brown.
1H. Perigynia distinctly nerved 31. *C. neurochleana*
2H. Perigynia almost nerveless 30. *C. heleonastes*
2G. Culms glabrous or nearly so, scales darker.
1H. Perigynia many-nerved, 1.5 mm. wide 32. *C. pribylovensis*
2H. Perigynia few-nerved, narrow 33. *C. glareosa*
2E. Spikes four to eight, the lower ones distant.
1F. Beaks of the perigynia scabrous on the margins.
1G. Beak and part of perigynia. with a distinct hyaline suture38. *C. brunnescens*
2G. Beak and perigynia without such suture.
1H. Perigynia 1.5–1.8 mm. long 37. *C. bonanzensis*
2H. Perigynia 2–3 mm. long..35. *C. canescens*
2F. Beaks of perigynia smooth.
1G. Perigynia about 3 mm. long..34. *C. mackenziei*
2G. Perigynia much smaller 36. *C. lapponica*
2D. Plants loosely tufted, stolons present.
1E. Spikes androgynous 39. *C. disperma*
2E. Spikes gynaecandrous.
1F. Spikes aggregated at top of culm 40. *C. tenuiflora*
2F. Spikes at some distance from each other 41. *C. loliacea*
2C. Perigynia not white-puncticulate, beaks long.
1D. Perigynia broadest near base.
1E. Perigynia 2.5–4 mm. long 42. *C. stellulata*

2E. Perigynia 4–4.5 mm. long 43. *C. phyllomanica*
2D. Perigynia tapering toward base 44. *C. laeviculmis*

Eucarices distigmaticae

1A. Beaks with truncate mouths 98. *C. physocarpa*
2A. Perigynia short-beaked or beakless.
1B. Lowest bract long-sheathing.
1C. Sheath 2–4 mm. long with black auricles..45. *C. bicolor*
2C. Sheaths longer, without black auricles.
1D. Perigynia white-papillose, dry 47. *C. garberi*
2D. Perigynia not or only slightly papillose, fleshy 46. *C. aurea*
2B. Lowest bract sheathless or nearly so.
1C. Lowest bract shorter than the inflorescence.
1D. Aphyllopodic, runners present 48. *C. bigelowii*
2D. Phyllopodic, runners absent 49. *C. lugens*
2C. Lowest bract as long as the inflorescence or longer.
1D. Spikes ovate, congested at the top of the culm 65. *C. enanderi*
2D. Spikes cylindrical or prolonged, the upper ones staminate.
1E. Culms with lower leaves blade-bearing (phyllopodic).
1F. Perigynia nerved, ovate.
1G. Scales acute, spikes slender..50. *C. kelloggii*
2G. Scales blunt, spikes thicker..51. *C. hindsii*
2F. Perigynia rounded.
1G. Scales strongly nerved 52. *C. kokrinensis*
2G. Scales not strongly nerved.53. *C. aquatilis*
2E. Culms with lower leaves not blade-bearing (aphyllopodic).
1F. Normally high-growing plants.
1G. Pistillate spikes usually erect, long and narrow 54. *C. sitchensis*
2G. Pistillate spikes drooping, rather short and thick 57. *C. lyngbyei*
2F. Comparatively low-growing, 3 dm. tall or less.
1G. Low-growing, spikes few-flowered 55. *C. subspathacea*
2G. Medium-low, spikes many-flowered 56. *C. ramenskii*

Eucarices tristigmaticae

1A. Beaks with truncate mouths.
1B. Bracts sheathless or nearly so.
1C. Lower bract foliaceous.
1D. Terminal spike staminate.
1E. Spikes more or less approximate....60. *C. stylosa*

2E. Spikes distant.
1F. Scales long-aristate 68. *C. macrochaeta*
2F. Scales short-aristate 84. *C. magellanica*
3F. Scales blunt or acute.
1G. Perigynia ciliate on the margins.
1H. Pistillate scales cuspidate 69. *C. karaginensis*
2H. Pistillate scales merely acute 88. *C. atrofusca*
2G. Perigynia smooth on the margins.
1H. Culms aphyllopodic.
1I. Scales with midveins reaching the apex and sometimes excurrent..71. *C. spectabilis*
2I. Scales with midveins obsolete toward the apex 70. *C. montanensis*
2H. Culms phyllopodic.
1I. Spikes linear, long and narrow 72. *C. nesophila*
2I. Spikes oblong, thick and short 73. *C. podocarpa*
2D. Terminal spike gynaecandrous.
1E. Pistillate scales awned or cuspidate.
1F. Spikes sessile 59. *C. buxbaumii*
2F. Spikes distinctly peduncled.....61. *C. gmelini*
2E. Pistillate scales not awned or cuspidate.
1F. Perigynia 5 mm. long, spikes six to ten 67. *C. mertensii*
2F. Perigynia shorter, spikes three to six.
1G. Perigynia nerved, sparsely spinulose on margins 65. *C. enanderi*
2G. Perigynia not spinulose on margins.
1H. Rootstocks long, leaves smooth 62. *C. leiophylla*
1I. Scales purplish-black with conspicuous hyaline margins.
1J. Culms slender, perigynia subinflated 58. *C. norvegica*
2J. Culms stiff, perigynia flat 63. *C. albo-nigra*
2I. Scales lacking distinct hyaline margins.
1J. Culms scabrous, spikes linear 66. *C. atratiformis*
2J. Culms glabrous, spikes oblong--ovoid 64. *C. atrata*
2C. Lower bract scale-like.
1D. Perigynia glabrous 77. *C. supina*

2D. Perigynia pubescent.
1E. Lower pistillate spikes on elongated subradical peduncles.
1F. Loosely caespitose, rootstocks thin 74. *C. deflexa*
2F. Densely caespitose, rootstocks stout 75. *C. rossii*
2E. Subradical pistillate spikes absent.. 76. *C. peckii*
2B. Bracts with distinct sheaths.
1C. Perigynia pubescent 78. *C. concinna*
2C. Perigynia glabrous
1D. Leaves 0.2–1 mm. wide, canaliculate or involute.
1E. Lowest bract bladeless 80. *C. eburnea*
2E. Lowest bract with a setaceous blade 79. *C. glacialis*
2D. Leaves broader, flat (or canaliculate).
1E. Pistillate spikes more or less densely flowered, drooping.
1F. Sheath of lowest bract long, tubiform 85. *C. laxa*
2F. Sheath of lowest bract short, spathiform.
1G. Lowest bract leaf-like.
2G. Lowest bract subulate.
1H. Pistillate scales obtuse.
1I. Pistillate spikes two to ten-flowered 81. *C. rariflora*
2I. Pistillate spikes ten to twenty-five flowered.. 82. *C. pluriflora*
2H. Pistillate scales cuspidate or mucronate 83. *C. limosa*
2E. Pistillate spikes loosely flowered, erect.
1F. Perigynia nearly beakless 86. *C. livida*
2F. Perigynia long-beaked 87. *C. vaginata*
2A. Beak with bidentate mouth, the teeth sometimes rather indistinct.
1B. Leaves not septate-nodulose.
1C. Perigynia flat, ciliate-serrulate on the margins.
1D. Perigynia rounded at the base, about as long as the scales.
1E. Pistillate scales cuspidate 69. *C. karaginensis*
2E. Pistillate scales merely acute 88. *C. atrofusca*
2D. Perigynia tapering at the base, longer than the scales 89. *C. misandra*
2C. Perigynia trigonous, not serrulate or ciliate on the margins.
1D. Spikes on capillary peduncles, drooping.
1E. Terminal spike gynaecandrous 91. *C. krausei*
2E. Terminal spike staminate.
1F. Leaves setiform, involute 92. *C. williamsii*
2F. Leaves flat 90. *C. capillaris*
2D. Spikes short on short peduncles, erect.

1E. Beak of perigynia as long as the body, curved 93. *C. flava*
2E. Beak of perigynia short, erect 94. *C. oederi*
2B. Leaves septate-nodulose.
1C. Teeth of beak subulate, 1 mm. long.....100. *C. atherodes*
2C. Teeth of beak shorter.
1D. Pistillate spikes 1–2 cm. long.
1E. Leaves flat, channeled toward the base 99. *C. membranacea*
2E. Leaves involute 96. *C. rotundata*
2D. Pistillate spikes 5–7 cm. long.
1E. Perigynia horizontal 97. *C. rhyncophysa*
2E. Perigynia ascending 95. *C. rostrata*

1. *C. nardina* Fr. Hepburn Sedge
C. hepburnii Boott.

Densely caespitose; culms 2–15 cm. tall, slender, wiry, not exceeding the leaves; leaves setaceous, stiff, erect or recurving, about 0.25 mm. wide; spike 5–15 mm. long, bractless; scales reddish-brown with straw-colored center; perigynia five to fifteen, 3.5–4.5 mm. long, biconvex or plano-convex, light-colored with some brown at the apex, sharp-edged, serrulate above; achenes lenticular or triangular, brown, apiculate; stigmas two or three.

Central Alaska—Alta.—Colo.—Wash. (Fig. 211.)

2. *C. jacobi-peteri* Hult. Anderson Sedge

Plants caespitose; culms 3–10 cm. tall, usually curved; leaves longer than the culm, flat, 1–1.5 mm. wide, usually curved; spikelet 4–11 mm. long, without bracts; scales acute or acuminate, brownish with greenish midrib, as long or nearly as long as the perigynia; perigynia decidedly stipitate, about 2.5 mm. long, brownish at tip; achenes lenticular, about 1.5 mm. long.

Known only from Tin City. (Fig. 212.)

3. *C. capitata* L. Capitate Sedge

Loosely caespitose; rootstocks slender, ascending obliquely, culms 10–35 cm. tall, erect; leaf-blades 0.5 mm. or less wide, filiform, involute; spike 4–10 mm. long, bractless; scales brown with hyaline apex and margins, the staminate narrower, more acute and lighter colored; perigynia six to twenty-five, 2–3 mm. long, plano-convex, sharp-edged, broad-margined; achenes yellowish-brown, lenticular.

Bering Strait region through central Alaska. Distribution circumpolar and in S. Am. (Fig. 213.)

4. *C. gynocrates* Wormskj. Northern Bog Sedge

Stoloniferous, stolons long, 1 mm. thick; culms 4–30 cm. long, stiff, obtusely triangular; leaves 0.5 mm. wide, involute, stiff; spike staminate, pistillate or androgynous, 5–15 mm. long, brownish with hyaline margins;

perigynia four to ten, 3–3.5 mm. long, ascending, spreading, or reflexed, often curved toward the tip, yellowish or dark, finely nerved dorsally, serrulate above, hyaline at the mouth; achenes lenticular, 1.5 mm. long, yellowish-brown, shining.

Throughout most of our area—Greenl.—N. Y.—Colo.—B. C.—Siberia. (Fig. 214.)

5. *C. scirpoidea* Michx. Northern Single-spike Sedge
C. stenochleana (Holm.) Mack.

Rootstocks creeping, dark reddish-purple; culms 1–5 dm. tall, stiff, roughened above; leaves 1–3 mm. wide, flat or canaliculate; spike dioecious, 1–3 cm. long, 3–7 mm. thick, often with a leaf-like bract 3–50 mm. long 5–50 mm. below the spike; pistillate scales brownish or blackish, with hyaline margins and lighter center, often more or less hairy on the back and with ciliate margins; perigynia compressed-triangular, dark-colored, short white-pubescent; achenes 1.5–2 mm. long, yellowish-brown.

A variable species of circumpolar distribution found throughout our area. (Fig. 215.)

6. *C. anthoxanthea* Presl.

Rootstocks rather long, scaly; culms 5–35 cm. tall, roughened above; leaves 1.5–2.5 mm. wide, erect or recurved; spike usually pistillate, sometimes androgynous or staminate, bractless; lower scales cuspidate or awned, the upper obtuse, chestnut-brown with one- to three-nerved lighter or greenish center; perigynia four to fourteen, about 4 mm. long, 1.5 mm. wide, compressed triangular, yellowish-green, many-nerved, achenes about 1.5 x 1 mm., triangular.

Grassy banks, Aleutian and Pribylof Islands.—B. C. (Fig. 216.)

7. *C. circinata* C. A. Mey. Coiled Sedge

Densely caespitose; culms 5–20 cm. long, erect or more often recurved; leaves about 0.5 mm. wide, involute-filiform, curved, stiff; spike androgynous, 15–30 mm. long, bractless; lowest scale cuspidate, the upper obtuse, reddish-brown with hyaline apex and margins; perigynia 4.5–6 mm. long, narrow, erect-ascending, obscurely compressed-triangular, straw-colored with some reddish-brown below the hyaline-tipped beak, serrulate; achene 2.5–3 mm. long, obtusely triangular.

Near the coast, Aleutian Islands—Wash. (Fig. 217.)

8. *C. leptalea* Wahl. Bristle-stalked Sedge

Caespitose; culms filiform, 1–5 dm. tall; leaves very narrow; spike androgynous, 4–15 mm. long, 2–3 mm. thick, bractless; staminate flowers few–many, their scales connate below; perigynia one to ten, 2.5–5 mm. long, thick, yellowish or light green, striate; lowest scale cuspidate, the upper usually obtuse; achenes 1.5–2 mm. long, triangular with concave sides below, yellowish or brownish, shining.

Bogs, central Alaska—Labr.—Fla.—Texas—Colo. (Fig. 218.)

9. *C. filifolia* Nutt. Thread-leaved Sedge

Densely caespitose, culms slender, stiff, 8–30 cm. tall; leaves acicular, involute, stiff, 3–20 cm. long, 0.25–0.5 mm. wide; spike 1–3 cm. long, bractless, the upper half staminate; scales usually obtuse, light reddish-brown with broad hyaline margins; perigynia five to fifteen, 3–35 mm. long, obtusely triangular, dull whitish or straw-colored, darker above, obscurely 2-ribbed; beak truncate, hyaline; achenes 2.25–3 mm. long, triangular.

Yukon—Man.—N. Mex.—eastern Ore.

10. *C. rupestris* All. Rock Sedge

Loosely caespitose and stoloniferous; culms 4–15 cm. tall, wiry; leaves 1–3 mm. wide, spreading or recurving, canaliculate, stiff; spike 1–2 cm. long, bractless; scales thin, chestnut-brown with hyaline margins and lighter center; perigynia three to eight, 3–4 mm. long, triangular, greenish straw-colored tinged brownish, shining, two-keeled; achenes 2.25 mm. long, triangular, dark chestnut-brown, short-apiculate.

Alpine-arctic; circumpolar. Rare in our area.

11. *C. obtusata* Lilj.

Rootstocks long-creeping, slender, purplish-black; culms 6–20 cm. tall, scattered, or two or three together; leaves channeled, 1–1.5 mm. wide; spike 5–12 mm. long, bractless; scales acuminate or cuspidate, thin, light brownish with hyaline margins and lighter midvein; perigynia one to six, 3–3.5 mm. long, dark chestnut or blackish brown, shining; beak obliquely cut, bidentulate, hyaline-tipped; achene 1.75 mm. long, triangular and with prominent ridges, light yellowish-brown.

Central Alaska—Man.—S. Dak.—N. Mex.—B. C.—Eurasia. (Fig. 219.)

12. *C. pyrenaica* Wahl. Pyrenean Sedge

C. pyrenaica Wahl ssp. *micropoda* (C. A. Mey) Hult.

C. micropoda C. A. Mey.

Densely caespitose; culms slender, 3–25 cm. tall; leaves 0.25–1.5 mm. wide, channeled; spike androgynous, 5–20 mm. long, 3–5 mm. thick, bractless; scales blackish-chestnut to straw-color, with hyaline margins; staminate flowers inconspicuous; perigynia ten to many, brownish above, lighter at the base, shining; achenes 1.25–1.5 mm. long, light brown.

Aleutian and Pribylof Islands eastward; circumboreal. (Fig. 220.)

13. *C. nigricans* C. A. Mey. Blackish Sedge

Loosely caespitose; culms 5–30 cm. tall, striate, rather stiff; leaves 1.5–2 mm. wide; spike androgynous, 8–15 mm. long, bractless; staminate scales persistent, reddish-brown, becoming straw-colored; pistillate scales deciduous, dark brown; staminate flowers conspicuous; perigynia several to fifty, 3.5–4 mm. long, exceeding the scales, jointed to the rachis, deflexed at maturity; compressed-triangular, yellowish to brownish, the orifice hyaline; achenes 1.5–2 mm. long, triangular, yellowish-brown.

Aleutian and Commander Islands—Colo.—Calif. (Fig. 221.)

14. *C. pauciflora* Lightf. Few-flowered Sedge

Rootstocks long, slender; culms 5–60 cm. tall, stiff; leaves 0.75–1.5 mm. wide, involute or channeled; spike androgynous, bractless; scales acutish, light-colored, the pistillate early deciduous; perigynia one to six, 6–7 mm. long, soon reflexed, light green, soon becoming straw-color or brownish, finely striate; achene about 2 mm. long, triangular with concave sides near the base, usually convex above; stigmas three, short.

Muskegs, Pacific Coast of Alaska; circumboreal. (Fig. 222.)

15. *C. microglochin* Wahl. False Uncinia

Rootstocks long, slender; culms 5–25 cm. tall, stiff; leaves about 0.5 mm. wide, involute, light green with blunt tip; spike androgynous, 7–14 mm. long, bractless; scales light chestnut brown, sometimes with lighter margins and center; perigynia three to twelve, 4–6 mm. long, about 1 mm. wide, bright brownish-green or straw-color, finally reflexed, the orifice oblique; achenes about 2.5 mm. long, triangular, yellowish-brown.

Bering Sea through central Alaska; circumpolar. (Fig. 223.)

16. *C. maritima* Gunner. Curved Sedge
C. incurva Lightf.

Rootstocks long, forking; culms solitary or a few together, 2–16 cm. long, stiff, usually more or less curved; leaves 2–10 cm. long, 1–2 mm. wide, involute above, erect or recurved-spreading; spikes four to twelve, in a dense head 6–12 mm. long, bractless; staminate flowers inconspicuous; perigynia 3.25–4 mm. long, longer than the scales, plano-convex, slightly inflated, shaded light yellowish-brown to brownish-black, sharp-edged, sparingly serrulate on and near the beak; achenes 1.5 mm. long, lenticular, brownish.

Near the coast and in tundra, arctic—southeastern Alaska; circumpolar. (Fig. 224.)

17. *C. stenophylla* Wahl ssp.*eleocharis* (Bailey) Hult. Involute-leaved Sedge
C. eleocharis Bailey.

Rootstocks long, slender, culms one or a few together, 3–20 cm. tall, slender, stiff; leaves 1–1.5 mm. wide, involute above; spikes few, aggregated into a head 7–15 mm. long; bracts ovate, cuspidate; scales slightly exceeding the perigynia, brownish with wide hyaline margins; perigynia one to eight to a spike, 2.5–3 mm. long, slightly elevated and serrulate near and along the beak; achenes lenticular, about 1.75 x 1.5 mm.

Yukon—Slave Lake—Man.—Iowa—N. Mex.—E. Ore. The full species is circumboreal.

18. *C. chordorrhiza* Ehrh. Creeping Sedge

Old culms prostrate, producing fertile culms 1–3 dm. tall terminally and from upper nodes, sterile culms from the lower nodes; leaves about 1 mm. wide, slightly scabrous, canaliculate; acute or acuminate, light brown with hyaline margins and lighter center; perigynia 2.5–3.5 mm.

long, concealed by the scales, thick plano-convex, brownish, shining, strongly nerved, sharp-edged; achenes lenticular, 1.75–2 mm. x 1.25 mm., thick, brownish, punctate.

Collected on Buckland River; circumboreal. (Fig. 225.)

19. *C. praegracilis* W. Boott. Clustered Field-sedge

Rootstocks long, stout, black; culms 20–75 cm. tall, roughened above; leaves 1.5–3 mm. wide, flat or channeled; spikes five to fifteen, 4–8 mm. x 4–6 mm. in a head 1–5 cm. long; bracts none or one or two; scales acute or cuspidate, nearly concealing the perigynia, dull brownish with hyaline margins; perigynia plano-convex, smooth, dull blackish with age, 3–4 mm. long, 1.5 mm. wide, nerved dorsally, the margins sharp; beak about 1 mm. long, serrulate, obliquely cut and hyaline at orifice; achenes 1.25 mm. long, lenticular.

Yukon—Sask.—Man.—Kans.—Mex.—L. Calif.

20. *C. stipata* Muhl. Awl-fruited Sedge

Caespitose; culms 3–12 dm. tall, sharply triangular, erect, weak, flattened in drying; leaves 4–10 mm. wide, flat, flaccid, serrulate on the margins near the apex; spikes many, yellowish-brown, in a compound head 3–10 cm. long, 10–25 mm. thick; lowest bract setiform, up to 5 cm. long, or lacking; scales acuminate or cuspidate, brownish or hyaline; staminate flowers inconspicuous; perigynia 4–5 mm. long, plano-convex, thick, yellowish, strongly nerved, sharp-edged; beak 2–2.5 mm. long, serrulate, tipped reddish-brown; achenes 1.5–2 x 1.25–1.75 mm., plump.

Eastern Asia—coast of Alaska—Newf.—N. C.—Calif. (Fig. 226.)

21. *C. diandra* Schk. Lesser Panicled Sedge

Caespitose; culms 3–7 dm. tall, stiff, roughened on the edges; leaves 1–2.5 mm. wide; spikes many, in a brownish head 2–5 cm. long; bracts short, subulate, often absent; scales acute or cuspidate, brownish with hyaline margins and lighter midrib; staminate flowers inconspicuous; perigynia 2–2.75 mm. long, strongly biconvex, brown, shining, few-nerved dorsally, sharp-edged and serrulate above; achenes lenticular.

Wet meadows, central Alaska east and south. Circumboreal and in New Zealand. (Fig. 227.)

22. *C. macrocephala* Willd. ssp. *anthericoides* (Presl.) Hult. Large-headed Sedge

Perpendicular rootstocks from long, deep, horizontal ones; culms 15–35 cm. tall, stiff, stout; leaves 4–8 mm. wide, the margins minutely serrulate; heads 4–6 x 2.5–5 cm., composed of numerous scarcely distinguishable spikes about 1.5 cm. long; bracts variable, sometimes highly developed; scales acuminate to awned, striate, brownish with green center and hyaline margins; perigynia 10–15 x 4–6 mm., thick, shining, strongly nerved, the margins winged and serrulate; beak 4–7 mm. long, bidentate; achenes 4 x 2.5 mm., triangular, constricted in the middle.

Along the coast, Alaska—Calif. Main species in eastern Asia.

23. *C. athrostachya* Olney. Slender-beaked Sedge

Caespitose with short rootstocks; culms 5–60 cm. tall; leaves 1.5–3 mm. wide; spikes 4–20, ovoid, 4–10 mm. long, in a head 1–3 cm. long; lower bracts elongated and exceeding the head, dilated and hyaline-margined at the base; scales acute to cuspidate, brownish with hyaline margins and green center; staminate flowers inconspicuous; perigynia ascending, ovate-lanceolate, thin, 3–4 mm. long, wing-margined, serrulate above; achenes lenticular; stigmas two.

Southeastern Alaska—Sask.—Colo.—Calif. (Fig. 228.)

24. *C. macloviana* d'Urv. ssp. *pachystachya* (Cham.) Hult. Thick-headed Sedge

C. pachystachya Cham.

Densely caespitose, culms 3–10 dm. tall, striate; leaves 2–4 mm. wide, flat; spikes four to twelve, 5–8 x 4–6 mm., in a dense head 10–25 mm. long; bracts scale-like or the lower awned; scales acute, brown or blackish, often with lighter midrib; staminate flowers inconspicuous; perigynia six to twenty to a spike, 4.5–6.5 mm. long, appressed, plano-convex, wing-margined, serrulate, light-colored; beak brownish, bidentulate; achenes 1.5–2 mm. long, lenticular, yellowish-brown; stigmas two.

Central Alaska—Greenl.—Que.—Colo.—Calif. (Fig. 229.)

25. *C. praticola* Rydb. Meadow Sedge

Caespitose; culms 2–7 dm. tall; leaves 1–3.5 mm. wide, flat, light green; spikes two to seven, 6–16 x 4–6 mm., in a flexuous head 15–50 mm. long; bracts scale-like, the lowest often cuspidate; scales acutish,, tinged reddish-brown with silvery-hyaline margins; staminate flowers inconspicuous; perigynia six to twenty to a spike, 4.5–6.5 mm. long, appressed, plano-convex, wing-margined, serrulate, light-colored; beak brownish, bidentulate; achenes 1.5–2 mm. long, lenticular, yellowish-brown; stigmas two.

Central Alaska—Greenl.—Que.—Colo.—Calif. (Fig. 230.)

26. *C. phaeocephala* Piper. Mountain Hare Sedge

Caespitose with densely matted rootstocks; culms 1–3 dm. tall, stiff; leaves 1.5–2 mm. wide, canaliculate or involute; spikes two to five, occasionally up to seven, 6–12 x 5–8 mm., in a head 12–25 mm. long; lowest bract sometimes developed; scales acute, covering the perigynia, dark with hyaline margins and lighter midvein; staminate flowers conspicuous; perigynia 4–6 mm. long, oblong-ovate, plano-convex, brownish, strongly veined dorsally, wing-margined, minutely serrulate; beak 1 mm. long; achenes 1.5 x 1 mm., lenticular, brownish.

Reported from Glacier Bay—B. C.—Alta.—Colo.—Calif.

27. *C. crawfordii* Fern. Crawford Sedge

Densely caespitose; culms 1–6 dm. tall, stiff; leaves 1–3 mm. wide; spikes three to twelve, densely-flowered, in a head 12–25 mm. long; lower bracts setaceous; scales acute or acuminate, light brown with greenish

center; staminate flowers inconspicuous; perigynia numerous, about 4 mm. long, thin, distended over the achene, brownish, winged, serrulate above; beak bidentate, reddish-brown at the tip; achenes about 1 mm. long, lenticular with prominent beak.

Central Alaska—Newf.—N. Jer.—Mich.—Wash. (Fig. 231.)

28. *C. aenea* Fern. Fernald Hay-Sedge

Caespitose; culms 3–12 dm. tall, nodding; leaves 2–4 mm. wide, flat, weak; spikes four to ten, 7–25 x 5–7 mm., in a flexuous, moniliform or loose head 35–70 mm. long; lower bracts cuspidate, the upper scale-like; scales acute or acuminate, dull or yellowish brown with hyaline margins and three-ribbed green center; perigynia 4–5 x 2 mm., nearly concealed by the scales, concavo-convex, dull green or brownish, nerved dorsally, delicately serrulate above; achenes 2 x 1.5 mm., dull yellowish-brown.

Circle Hot Springs—Labr.—Newf.—N. Y.—S. Dak.—B. C. (Fig. 232.)

29. *C. lachenalii* Schk. Arctic Hare's-foot Sedge
C. bipartata All.
C. lagopina Wahl.

Loosely caespitose, rootstocks short, brownish; culms 5–30 cm. tall, slender, erect or curving; leaves 1–3 mm. wide; spikes two to five, dark brown, 5–10 mm. long in a head 1–2 cm. long; bracts scale-like; scales obtuse, keeled, chestnut-brown with hyaline margins and yellowish-brown center; perigynia 2–3.5 mm. long, appressed-ascending, plano-convex, several-nerved, sharp-edged; achenes about 1.5 mm. long.

Arctic-alpine situations; circumpolar. (Fig. 233.)

30. *C. heleonastes* Ehrh. Hudson Bay Sedge

Loosely caespitose with long slender rootstocks; culms slender, stiff, 15–35 cm. tall; leaves 1–2 mm. wide, flat or involute; spikes two to four, 4–7 x 4–6 mm. in a head 8–18 mm. long; bract scale-like, sometimes cuspidate; scales thin, keeled, reddish-brown with narrow hyaline margins and lighter center; perigynia five to ten to a spike, 2.5–3 mm. long, 1.25 mm. wide, plano-convex, thick, blunt-edged, faintly nerved; beak 0.5 mm. long, cleft dorsally; achenes lenticular, 1.5 x 1 mm.

Known from Kusilof; circumboreal but local.

31. *C. neurochlaena* Holm. Northern Clustered Sedge

Caespitose in small clumps; rootstocks very slender; culms scabrous, slender, weak, often curved, 15–25 cm. long; leaves canaliculate, 0.75–1.5 mm. wide; spikes two to four, the terminal one gynaecandrous, 7–12 mm. long, the lower usually pistillate and shorter; scales distinctly hyaline-margined; perigynia distinctly nerved.

Yukon and N. W. Territories.

32. *C. pribylovensis* Macoun. Pribylof Sedge

Loosely caespitose; culms 2–4 dm. tall, stiff or slightly flexuous; leaves 1–2.5 mm. wide, flat, thickish; spikes three to four, the terminal gynaecan-

drous, 7–12 mm. long, the lateral usually pistillate and shorter, in a head 12–20 mm. long; scales keeled, deep brown with wide hyaline margins and straw-colored center; perigynia ten to thirty to a spike, 2.5–3 x 1.5 mm., light yellowish-green; achenes lenticular, about 2 mm. long.

Aleutian Islands and islands in Bering Sea. (Fig. 234.)

33. *C. glareosa* Wahl. Weak Clustered Sedge

Loosely caespitose; rootstocks long, slender; culms 10–25 cm. tall, smooth, brownish; leaves 0.5–1.5 mm. wide, canaliculate; spikes two or three, the terminal gynaecandrous, 7–12 mm. long, 2 mm. wide, the lateral pistillate and shorter, in a head 12–19 mm. long; bracts usually scale-like; scales thin, keeled, brownish with hyaline margins; perigynia narrow, about 3.5 mm. long, plano-convex, brownish above, lighter below; achenes lenticular, nearly filling the perigynia.

Coastal regions; circumpolar. (Fig. 235.)

34. *C. mackenziei* Kretch. Norway Sedge
C. norvegica Willd. not Retz.

Rootstocks long, slender; culms 10–45 cm. tall, smooth; leaves 1–3 mm. wide, flat, thin, soft, yellowish-green; spikes three to six, the terminal gynaecandrous, 1–2 cm. long, the lateral gynaecandrous or pistillate, 5–15 mm. long, in a head 15–55 mm. long; bracts scale-like, the lowest often setaceous-pointed; scales light reddish-brown with hyaline margins and lighter center; perigynia five to twenty to a spike 2.5–3.3 mm. long, plano-convex, thick, glaucous-green, white-puncticulate, striate; achenes about 2 mm. long, lenticular, filling the perigynia.

Along the coast; circumboreal. (Fig. 236.)

35. *C. canescens* L. Silvery Sedge

Caespitose, the rootstocks short; culms 2–8 dm. tall, erect; leaves flat, 2–4 mm. wide, shorter than the culm; spikes four to eight, 3–12 mm. long, in a cluster 2–15 cm. long; bracts scale-like, the lowest often prolonged into a bristle; scales hyaline with greenish center and somewhat brown-tinged when mature; perigynia 1.8–2.8 mm. long, plano-convex, gray-green or yellowish-brown, white-puncticulate, rough or minutely serrulate near the apex, brownish-tinged at mouth; achenes lenticular, 1.5 mm. long, yellowish-brown.

Common in swamps and bogs; circumboreal. (Fig. 237.)

36. *C. lapponica* O. F. Lang. Lapland Sedge

Resembles *C. canescens* but is less distinctly tufted, the culms and leaves are more slender, the spikes are smaller and fewer-flowered, and the perigynia are smooth and not serrulate on the margins.

Bering Sea and central Yukon regions; circumpolar and more arctic in distribution than *C. canescens*.

37. *C. bonanzensis* Britt. Yukon Sedge

Caespitose; rootstocks long, slender; culms 25–45 cm. tall, stiff, with concave sides; leaves 2–3 mm. wide, flat; spikes about seven, 5–14 x 4

mm., the lower distant; lowest bract 15–30 mm. long, the upper scale-like; scales thin, keeled; staminate flowers conspicuous in terminal spike; perigynia small, about 1.5 x 1 mm., exceeding the scales, dark straw-colored, white-puncticulate, strongly nerved dorsally, sharp-edged; achenes about 1.25 mm. long.

Yukon and Siberia.

38. *C. brunnescens* (Pers.) Poir. Brownish Sedge

Caespitose, rootstocks short; culms 7–70 cm. tall, slender, lax; leaves long, 1–2.5 mm. wide, roughened toward the apex; spikes five to ten, mostly gynaecandrous, scattered, the lateral 3–7 mm. long, the terminal up to 13 mm. long; lowest bract prolonged, the upper scale-like; scales white-hyaline with greenish center and usually more or less tinged with brown; perigynia 2–2.5 mm. long, appressed-ascending, plano-convex, greenish or brownish, puncticulate, nerved, finely serrulate above; beak bidentate; achenes lenticular.

Pacific coastal and Alaskan Range districts; circumboreal. (Fig. 238.)

39. *C. disperma* Dewey. Soft-leaved Sedge

Loosely caespitose; culms 1–6 dm. tall, slender, weak; leaves 0.75–2 mm. wide, soft, thin; spikes two to four, in a cluster 15–25 mm. long; bracts setaceous, less than 1 cm. long; scales acuminate or mucronate, white-hyaline with greenish midrib; staminate flowers one or two, inconspicuous, perigynia one to six to a spike, 2.25–3 mm. long, biconvex, light or yellowish-green, often darker with age, finely nerved; achenes about 1.75 mm. long, lenticular, brownish-yellow, shining, filling the perigynia.

Yukon valley and Pacific coastal districts; circumboreal. (Fig. 239.)

40. *C. tenuiflora* Wahl. Sparse-flowered Sedge

Loosely caespitose; culms 15–60 cm. tall, slender; leaves 0.5–2 mm. wide, soft, flat or canaliculate; spikes two to four, 4–9 x 3–6 mm., whitish, in a head 6–12 mm. long; bracts scale-like or the lower prolonged; scales obtuse with a three-nerved center; staminate flowers inconspicuous; perigynia three to fifteen in a spike, 3–3.5 mm. long, concealed by the scales, greenish-white, obscurely nerved; achenes about 2 x 1.5 mm., lenticular, light brown.

Sphagnum bogs, not common; circumboreal. (Fig. 240.)

41. *C. loliacea* L.

Loosely caespitose with long, slender stolons; culms 15–40 cm. long, slender, weak; leaves 0.5–2 mm. wide, flat, soft; spikes two to five, in a cluster 1–3 cm. long, lowest bract up to 8 mm. long, the upper scale-like; scales keeled, thin, hyaline; staminate flowers inconspicuous; perigynia three to eight to a spike, 2.5–3 mm. long, thick, plano-convex, light green, white-puncticulate, finely ribbed, beakless; achenes lenticular, 1.75 mm. long.

Central Alaska—Alta.—B. C.—also Eurasia. (Fig. 241.)

42. *C. stellulata* Good. Little Prickly Sedge

Caespitose; culms wiry, 15–35 cm. tall; leaves 1–2 mm. wide, flat or canaliculate; spikes two to four, the terminal gynaecandrous, the lateral

usually pistillate, in a head 1–3 cm. long; lower bract often cuspidate, the upper scale-like; scales light brown with wide hyaline margins and green midrib; perigynia three to ten to a spike, 2.5–3.25 mm. long, exceeding the scales, spreading, thick, nerved dorsally, sharp-edged, serrulate toward and on the bidentate beak; achene about 1.5 mm. long, lenticular, yellowish-brown. Reports of *C. echinata* Murr., *C. leersii* Willd. and *C. muricata* L. from Alaska all refer to this species.

Aleutian Islands; probably more or less circumboreal. (Fig. 242.)

43. *C. phyllomanica* W. Boott. Coastal Stellate Sedge

Caespitose from slender creeping rootstocks; culms 2–6 dm. tall; leaves 1.75–2.75 mm. wide, flat or canaliculate; spikes three to four, bur-like, in a head 15–25 mm. long, the terminal gynaecandrous, the lateral often pistillate; lowest bract setaceous, the upper scale-like; scales obtuse, light brown with hyaline margins and green center; perigynia eight to fifteen to a spike, 3.75–4.5 mm. long, plano-convex, thick, light-colored, striate, tapering into a serrulate beak; achenes about 2 mm. long, yellowish.

Southern Alaska—Calif. (Fig. 243.)

44. *C. laeviculmis* Meinsh. Smooth-stemmed Sedge

Caespitose with short, slender rootstocks; culms slender, 3–7 dm. tall; leaves 1–2 mm. wide, flat, weak; spikes three to eight, the terminal gynaecandrous, the lateral pistillate, the upper approximate, the lower distant, 3–10 mm. long; lowest bract 15–50 mm. long, the upper reduced; scales ovate, hyaline with conspicuous green midrib; perigynia three to ten to a spike, 2.5–4 mm. long, light or yellowish-green, sharp-edged, serrulate above, tawny-tipped; achenes 1.25–1.75 mm. long, lenticular, brownish.

Seward Peninsula—Mont.—Calif. (Fig. 244.)

45. *C. bicolor* All. Two-color Sedge

Loosely caespitose and stoloniferous; culms 5–20 cm. tall, roughened above; leaves 3–6 cm. long, 1–2.5 mm. wide; spikes two to five, the terminal gynaecandrous, the lateral pistillate, 5–10 mm. long; lower bract short-sheathing, leaf-like; the upper scale-like; scales obtuse or mucronate, dark with yellowish-green center; perigynia 2–2.5 long, appressed-ascending, glabrous, glandular-roughened, ribbed; achenes lenticular, yellowish-brown, puncticulate.

Southern half of our area; circumboreal. (Fig. 245.)

46. *C. aurea* Nutt. Gold-fruited Sedge

Loosely caespitose and stoloniferous; culms 5–55 cm. tall; leaves 2–4 mm. wide; terminal spike staminate, 3–10 mm. long, occasionally with a few perigynia; lateral spikes three to five, pistillate, 4–20 mm. long, the lowest on nearly basal peduncles 3–8 cm. long; bracts leaf-like, sheathing; scales light reddish-brown with hyaline margins and a wide light center; perigynia 2–3 mm. long, flattened-oval, translucent, fleshy, puncticulate, coarsely ribbed; achenes lenticular, brownish, minutely puncticulate.

Central Alaska—Newf.—Pa.—Nebr.—N. Mex.—Calif. (Fig. 246.)

47. *C. garberi* Fern. ssp. *bifaria* Fern. Garber Sedge
C. hassei Am. auct.

Loosely caespitose and stoloniferous; culms 5–70 cm. tall; leaves 2–4 mm. wide, flat above, channeled below; terminal spike gynaecandrous or staminate, 6–20 mm. long; lateral spikes three to five, pistillate, 7–25 mm. long, the lower on long, rough peduncles; lower bracts short-sheathing; scales brown with hyaline margins and prominent light center; perigynia 2.5–3 mm. long, flattened-suborbicular, whitish, minutely granular; achenes lenticular, 1.5 mm. long, brown, puncticulate.

Southern half of our area—Alta.—B. C. also near mouth of St. Lawrence R. (Fig. 247.)

48. *C. bigelowii* Torr. Bigelow Sedge
C. concolor R. Br.

Stoloniferous, the stolons horizontal or ascending; culms 1–4 dm. tall, rather stout and stiff; leaves 2–8 mm. wide, flat; terminal spike staminate, 5–25 mm. long; lateral spikes one to six, pistillate or the upper androgynous, 5–30 mm. long; lowest bract leaf-like, black-auricled, the upper reduced and scale-like; scales brownish-black with narrow hyaline margins and lighter midrib; perigynia 2.5–3.5 mm. long, biconvex, light green, usually purplish-black blotched above, two-ribbed, short-beaked, the orifice entire; achenes lenticular, 1.5–2 mm. long.

Most of our area; circumboreal. (Fig. 248.)

49. *C. lugens* Holm.

Densely caespitose; culms 2–5 dm. tall; leaves 1–2.5 mm. wide, channeled and with revolute margins; terminal spike staminate, 10–25 mm. long; lateral spikes two to three, pistillate or occasionally one of them androgynous, 8–25 mm. long; lowest bract 5–30 mm. long, black-auricled, the upper reduced to auricles; scales blackish with lighter margins and midrib; perigynia 1.5–2.5 mm. long, appressed plano-convex, straw-color below, dark above, beak short, purple-black; achenes lenticular, dark, filling the perigynia.

Bering Sea region—Mack. (Fig. 249.)

50. *C. kelloggii* W. Bott. Kellogg Sedge

Caespitose with very short ascending stolons; culms slender, 2–7 dm. tall, leaves 1.5–3 mm. wide; terminal spike staminate, 1–4 cm. long; lateral spikes three to five, pistillate, 15–35 mm. long, lowest bract leaf-like, the upper reduced; scales dark with hyaline margins and light center; perigynia numerous, appressed-ascending, 1.5–3 mm. long, flattened biconvex, light green, granular, the beak usually black-tipped; achenes 1 mm. long, lenticular, blackish.

Pacific Coast regions—Alta.—Colo.—Calif. (Fig. 250.)

51. *C. hindsii* C. B. Clarke. Hinds Sedge

Caespitose with short or long branching rootstocks; culms 1–5 dm. tall; leaves 1.5–3 mm. wide; terminal spike staminate, 15–35 mm. long;

lateral spikes pistillate, 1–4 cm. long; lowest bract leaf-like, the upper reduced; scales purplish-black with lighter center; perigynia numerous, 2–3.5 mm. long, flattened biconvex, yellowish-green, ribbed, two-edged, papillate; beak usually black-tipped; achenes lenticular, brownish-black, 1.5 mm. long, granular.

Near the coast, Aleutian Islands—Calif. (Fig. 251.)

52. *C. kokrinensis* Porsild. Kokrines Mountain Sedge

Loosely caespitose; culms 25–35 cm. tall, erect, exceeding the leaves, somewhat flattened; leaves about 2 mm. wide, flat; spikes cylindrical, 1–2 cm. long, erect, usually four, the terminal gynaecandrous, the lateral pistillate but generally with a few staminate flowers at the apex, the upper three closely aggregated; uppermost bract equaling, the lower exceeding the inflorescence; scales black with conspicuous greenish mid-vein reaching to the apex; perigynia flattened on one side, nerveless, pale grayish-green, smooth; beak very short. May be a hybrid.

Kokrines Mountains.

53. *C. aquatilis* Wahl. Water Sedge

Rootstocks sending out long horizontal scaly stolons; culms caespitose, 3–7 dm. tall, slender, sharply triangular, reddened at the base; leaves 2–5 mm. wide; staminate spikes one or two, slender, 1–5 cm. long; pistillate or androgynous spikes two to four, sessile or short-peduncled, 1–4 cm. long; bracts leaf-like, the lower exceeding the culm; scales obtuse to acuminate, blackish or reddish-brown, one-nerved with light center; perigynia about 2.5 mm. long and half as wide, biconvex, puncticulate, glandular-dotted, two-ribbed; achenes lenticular, stigmas two.

A circumboreal species found in most of our area. (Fig. 252.)

54. *C. sitchensis* Prescott. Sitka Sedge

Caespitose; rootstocks short, creeping; culms 25–125 cm. tall, reddish-brown at base; leaves 3–9 mm. wide, flat with revolute margins or channeled toward the base; terminal one to four spikes staminate, 2–8 cm. long; lower three to five spikes pistillate or androgynous, 2–9 cm. long, erect or the lowest drooping on slender peduncles; bracts leaf-like; scales usually acute, longer than the perigynia; perigynia fifty to one hundred fifty to a spike, 2.5–3.5 x 1.25–2 mm., plano-convex, sharp-edged, achenes 1.5–2 mm. x 1 mm., brownish, loosely enveloped.

Along streams and lakes, southwestern Alaska—Calif. (Fig. 253.)

55. *C. subspathacea* Wormskj. Hoppner Sedge

Culms 3–20 cm. tall, stiff, smooth, usually curved, arising from elongated, horizontal rootstocks; leaves 1–2.5 mm. wide, flat, but involute toward the apex; lower bracts foliaceous, rather spathe-like; terminal spike staminate, 5–15 mm. long; lateral spikes pistillate, 5–15 mm. long; scales dark with hyaline margins and prominent light center; perigynia five to fifteen to a spike, about 3 mm. long, white puncticulate.

An arctic, circumpolar species of coastal marshes. (Fig. 254.)

56. *C. ramenskii* Komarov. Ramenski Sedge

Culms 1–3 dm. tall, stiff, from horizontal, creeping rootstocks; leaves 1.5–4 mm. wide, firm, flat or revolute toward the tip; upper one or two spikes staminate, 8–20 mm. long; lower two or three spikes pistillate or the upper of these androgynous, 1–3 cm. long; lower bracts leaf-like, scales dark, ovate, one-nerved, blunt; perigynia 2.5–3 mm. long, short-beaked or beakless, achenes about 2 mm. long, brownish. Var. *caudata* Hult. has the pistillate scales with awns 1–3 mm. long.

Coastal regions, Kenai Peninsula—Arctic and northeastern Asia. (Fig. 255.)

57. *C. lyngbyei* Hornem. ssp. *cryptocarpa* (C. A. Mey.) Hult. Lyngbye Sedge

C. cryptocarpa C. A. Mey.

Long stoloniferous; culms 2–10 dm. tall, purple-red or brownish at base; leaves 2–12 mm. wide, flat with revolute margins; upper one to three spikes staminate, lower two to four spikes pistillate or androgynous, 15–80 mm. long, many-flowered, pendulous on slender peduncles; lower bracts leaf-like, often exceeding the inflorescence, the upper reduced; scales lanceolate, acuminate, exceeding the perigynia, brownish or blackish with light center; perigynia 2.5–3.5 mm. long, biconvex, glaucous-green or brownish, puncticulate; achenes about 2.5 mm. long, lenticular.

Common in brackish soil along the coast except the high arctic. The species is circumboreal. (Fig. 256.)

58. *C. norvegica* Retz. ssp. *inferalpina* (Wahl.) Hult.

C. angarae Steud.

Caespitose; culms rather slender, 2–6 dm. tall; leaves flat, with roughened and often revolute margins, 2–4 mm. wide; spikes two to four, the terminal gynaecandrous, the lateral pistillate, 4–8 mm. long, 3–5 mm. thick; lower bract often leaf-like; scales ovate, 1.5–2.5 mm. long, dark with rather narrow hyaline margins; perigynia obtusely triangular, yellowish-green, 2–3 mm. long; achenes triangular, about 1.75 mm. long.

In all our area except the arctic. The species is circumboreal. (Fig. 257.)

59. *C. buxbaumii* Wahl. Buxbaum Sedge

Loosely caespitose with long, slender, horizontal stolons; culms 25–100 cm. tall; leaves 1.5–4 mm. wide, flat and keeled, with revolute margins and channeled toward the base; spikes two to five, the terminal gynaecandrous, 1–4 cm. long, the lateral pistillate, 5–20 mm. long; bracts dark-auricled, the upper reduced; scales acuminate or aristate, dark with light center; perigynia 2.5–4 mm. long, triangular-biconvex, glaucous-green, shaded brownish, papillose; achenes 1.75 x 1.5 mm., brownish, triangular with rounded angles.

Southern half of Alaska; circumboreal. (Fig. 258.)

60. *C. stylosa* C. A. Mey. Variegated Sedge

Caespitose; culms 15–50 cm. tall, slender; leaves 1.5–3 mm. wide, flat with revolute margins or channeled toward the base; terminal spike

staminate or with a few perigynia, 1–2 cm. long; lateral spikes two or three, pistillate, 7–18 mm. long; scales obtuse to acute, very dark, with hyaline margins and lighter midrib; perigynia 2.5–3.5 mm. long, yellowish-brown, tinged purplish-black, papillose above; achenes 1.5 x 1.25 mm., brownish, triangular.

Bering Strait—Greenl.—Newf.—Wash. Also eastern Asia. (Fig. 259.)

61. *C. gmelini* Hook. & Arn. Gmelin Sedge

Caespitose; rootstocks short, stout; culms 1–6 dm. tall, purplish-red at base; leaves 1.5–4 mm. wide, flat with revolute margins or channeled toward the base; spikes three to six, the terminal gynaecandrous or staminate, the lateral pistillate, 1–3 cm. long; lowest bract leaf-like, the upper reduced; scales dark with hyaline margins, light center and spiny-cuspidate tip; perigynia 4–5 mm. long, yellowish-brown, purple-tipped; achenes 1.75–2 mm. long.

Seashores, Norton Sound—B. C. and the Asiatic coast. (Fig. 260.)

62. *C. leiophylla* Mack. Carcross Sedge

Loosely caespitose; rootstocks long, slender; culms 25–35 cm. tall, nodding; leaves 2–3.5 mm. wide, flat or channeled and with revolute margins; spikes four or five, the terminal gynaecandrous, the lateral pistillate, in a dense head 25 mm. x 12–16 mm.; scales acute, purplish-brown, with slender midvein and hyaline margins at apex; perigynia ten to twenty to a spike, 4 x 2 mm., inflated, straw-colored blotched purple; achenes 2.25 x 1.5 mm., triangular.

Known only from Carcross, Yukon.

63. *C. albo-nigra* Mack. Black and White-scaled Sedge

Caespitose; culms 1–3 dm. tall, stiff; leaves 2.5–5 mm. wide, flat with revolute margins; spikes usually three, the terminal gynaecandrous, 10–12 mm. long, the lateral shorter and pistillate; lowest bract brownish-tinged and subsheathing at the base; scales purplish-black with white hyaline margins and apex; perigynia 3–3.5 x 2 mm., flattened, purplish-black, granular, the beak bidentate; achenes 1.25 x 0.75 mm., triangular with concave sides, light yellowish-brown.

Central Alaska and Wash.—Alta.—Colo.—Ariz.—Calif.

64. *C. atrata* L. Black-scaled Sedge

Caespitose; culms 15–50 cm. tall, usually nodding above; leaves 2–8 mm. wide; spikes three to seven, the terminal gynaecandrous, the lateral pistillate, 1–2 cm. long, the lower nodding on slender peduncles; lowest bract leaf-like, the upper reduced; scales obtuse to acute, mostly brownish-black with lighter midrib and often lighter margins and tip; perigynia appressed, flattened but distended by the achene, papillose, brown-spotted, the beak dark, emarginate; achenes about 2 mm. long, triangular, yellowish-brown. Ssp. *atrosquama* (Mack.) Hult. is the more common form. It has shorter scales and the beak of the perigynia is shorter and broader than the type.

A circumboreal arctic-alpine species found in central and southeastern Alaska. (Fig. 261.)

65. *C. enanderi* Hult. Enander Sedge

Loosely caespitose with long rhizomes; culms 10–25 cm. tall, stiff; leaves 1.5–2 mm. wide; spikes three to five, oblong, densely flowered, the lower long-peduncled; terminal spike gynaecandrous, the lateral pistillate; pistillate scales dark purplish without hyaline margins or tip but with conspicuous green midrib; perigynia densely puncticulate, distinctly nerved, sparsely ciliate-serrulate on the margins, almost beakless, stipitate; stigmas two or sometimes three.

Known from Skagway and Akutan.

66. *C. atratiformis* Britt. Black Sedge

Loosely caespitose; culms 2–10 dm. tall, roughened above; leaves 2.5–5 mm. wide, flat with revolute margins; spikes three to six, the terminal gynaecandrous, the lateral pistillate with occasionally a few staminate flowers at the base, 10–25 x 4–6 mm., the lower nodding on slender peduncles; scales acute to cuspidate, dark reddish-brown to black with hyaline margins; perigynia ten to thirty to a spike, 2.5–3 x 1.5–1.75 mm., flattened, purplish-brown or straw-colored below, puncticulate, the beak bidentate; achenes 1.5–1.75 x 0.75 mm., silvery-black, shining.

Yukon—Labr.—Newf.—Maine—Mich.—Alta.

67. *C. mertensii* Prescott. Mertens Sedge

Caespitose, with short stolons; culms 3–12 dm. tall, sharply triangular, rough; leaves 4–8 mm. wide, flat with revolute margins; spikes five to ten, 1–4 cm. long, the uppermost strongly staminate at the base, the lateral sparingly so, drooping on slender peduncles; the lower two or three bracts leaf-like; scales mostly acute, shorter than the perigynia, dark with light center; perigynia numerous, flat, thin, distended over the achene, light brownish, often dark-spotted near the apex, 4.5–6 mm. long; achenes triangular, silvery-brown, about 2 mm. long. Our most beautiful species of Carex.

Central Alaska—Mont.—Calif. Common in the coast regions. (Fig. 262.)

68. *C. macrochaeta* C. A. Mey. Alaska Long-awned Sedge

Loosely caespitose with densely matted rootstocks; culms 2–6 dm. tall, purplish-red at base; leaves 2–4 mm. wide, flat with revolute margins; terminal spike staminate, 15–25 mm. long; lateral spikes two to four, pistillate, 1–3 cm. long, erect or drooping on slender peduncles; lower bract leaf-like, the upper reduced; scales dark with light whitish midrib excurrent as a serrulate awn 2–12 mm. long; perigynia 4.5–6 mm. long, smooth, obscurely nerved, straw-color or blotched or brownish, the beak dark; achenes 2–2.5 mm. long, triangular, brownish.

Near the coast, Aleutian and Pribylof Islands—Calif.—eastern Asia. One of our commonest species. (Fig. 263.)

69. *C. karaginensis* Meinsh. Karaginsk Island Sedge

Caespitose and short-stoloniferous; culms 15–80 cm. tall, slender; leaves 3–5 mm. wide; terminal spike staminate, 15–20 mm. long; lateral

spikes pistillate; lower bracts foliaceous, the upper scale-like; scales oblong-lanceolate, obtuse or short-cuspidate, dark purplish-black or brownish; perigynia compressed-triangular, broad, the margins ciliate-serrulate, the beak emarginate or shallowly bidentate.

St. Matthew Island—northeastern Asia.

70. *C. montanensis* Bailey. Montana Sedge

Loosely caespitose; rootstocks long, slender; culms 1–5 dm. tall, stiff, somewhat nodding above; leaves 2–4 mm. wide, flat, firm; terminal spike staminate, 7–25 mm. long, sometimes with a smaller one at the base; lateral spikes two to four, pistillate or sometimes androgynous, 1–2 cm. x 4–6 mm., drooping or erect; bracts black-auricled; scales acute to obtuse, nearly black with inconspicuous or obsolete, lighter midrib; perigynia about 4 x 2 mm., glandular, straw-color with darker shadings; achenes 1.5 x 0.75 mm., brownish, triangular with concave sides, long-stipitate.

Eastern Asia across Alaska—Mack.—Mont.—Ida. (Fig. 264.)

71. *C. spectabilis* Dewey. Showy Sedge

Rootstocks stout, short-branching, matted; culms 25–90 cm. tall, slender; leaves 2–5 mm. wide, flat with revolute margins; terminal and occasionally the second spike staminate, 8–20 mm. long; pistillate spikes one to four, 1–4 cm. long; scales cuspidate, blackish with thick, lighter or whitish midrib, the pistillate with hyaline margins; perigynia 4–5 mm. long, flattened, light green, blotched, glandular-roughened; achenes 2.5 x 1 mm., triangular, light brown.

Asia—Bering Sea and Pacific regions of Alaska—Alta.—Mont.—Calif. (Fig. 265.)

72. *C. nesophila* Holm. Bering Sea Sedge

Stoloniferous, the stolons ascending; culms 1–4 dm. long, stiff; leaves 2.5–6 mm. wide, flat, with revolute margins, stiff; terminal spike staminate, 1–2 cm. long; lateral spikes two to five; pistillate 3–35 mm. long, erect on stiff peduncles; scales obtuse or acute to cuspidate, purplish-black with whitish midvein and occasionally hyaline margins or tip; perigynia 3–4.5 mm. long, flattened-triangular, light-colored with darker shadings, the faces three-nerved; achene nearly 2 mm. long, triangular, yellowish-brown.

Coast regions of Bering Sea—Kenai Peninsula. (Fig. 266.)

73. *C. podocarpa* R. Br. Short-stalk Sedge

Caespitose; culms 15–60 cm. tall; leaves 2.5–6 mm. wide, flat with revolute margins, the lower ones much reduced, bright green; terminal one or two spikes staminate, 1–3 cm. long; pistillate spikes two to six, 7–25 mm. long, 4-6 mm. thick; bracts dark-auricled, the upper reduced; scales usually acute, purplish-black with light midrib, some with hyaline margins; perigynia 2–4 mm. long, flattened, light-green, purplish-blotched, two-ribbed, the beak bidentulate; achenes 1.75 mm. long, triangular, light brown.

Central Alaska—Mack.—Mont.—Wyo.—Ore. (Fig. 267.)

74. *C. deflexa* Hornem. Northern Sedge

Caespitose; rootstocks branching, slender; culms 2–24 cm. tall, very slender; leaves short, 1–2 mm. wide, flat above, channeled toward the base, thin; terminal spike staminate, 2–5 x 0.5–1 mm.; lateral spikes two to four, pistillate, 2–6 x 3 mm., the lowest nearly basal on capillary peduncles; scales acute to cuspidate, brown with hyaline margins and lighter or green center; perigynia two to eight to a spike, about 2.5 x 1 mm., green, short-pubescent, ciliate-serrulate on the bidentate beak; achenes 1.5 mm. long, triangular.

Head of Yukon R.—L. Athabaska—Greenl.—Newf.—N. Y.—Mich.—Man. (Fig. 268.)

75. *C. rossii* Boott. Ross Sedge

Caespitose; culms 5–30 cm. tall, roughened above; leaves 1–2.5 mm. wide, channeled above, thin, firm; terminal spike staminate, 3–15 mm. long; lateral spikes two or three in the inflorescence near the top, usually with one or two from near the base on long slender peduncles, 3–5 mm. long; scales obtuse to cuspidate or awned; perigynia three to fifteen to a spike, 3–4.5 x 1 mm., pale green, short-pubescent, stipitate, with rather long ciliate-serrulate, bidentate beak; achenes triangular with concave sides.

Eastern Alaska and Yukon—L. Athabaska—Mich.—S. Dak.—Colo.—Calif. (Fig. 269) (from Colo.).

76. *C. peckii* Howe. Peck Sedge

Caespitose and stoloniferous; culms 15–65 cm. tall; leaves 1–1.5 mm. wide, flat; terminal spike staminate, 1–13 mm. long, inconspicuous; lateral spikes pistillate, 4–8 x 4 mm. in an inflorescence 8–20 mm. long; scales obtuse to mucronate, reddish-brown with hyaline margins, lighter center and green roughish midvein; perigynia three to twelve to a spike, about 3.5 x 1 mm., grayish- or yellowish-green, hirsute-pubescent, two-ridged, stipitate; beak obliquely cut, bidentulate; achenes about 2 x 1 mm., triangular with convex sides, yellowish-brown.

Yukon—Que.—N. B.—N. Jer.—Wis.—B. C.

77. *C. supina* Willd. ssp. *spaniocarpa* (Steud.) Hult. Weak Arctic Sedge
C. spaniocarpa Steud.

Caespitose and stoloniferous; culms 5–30 cm. tall; leaves 1–1.5 mm. wide, channeled, stiff, roughened, especially toward the attenuate apex; terminal spike staminate, 6–25 mm. long; lateral spikes one to three pistillate, 4–12 x 4 mm.; bracts scale-like; scales reddish-brown with wide hyaline margins and lighter center; perigynia four to fifteen to a spike or the one immediately below the terminal spike reduced to one to four, 2.5–3.5 mm. long, hard, brownish, shining; beak with hyaline orifice, achenes yellowish-brown, 2 x 1.5 mm.

Central Alaska—Baffin Land—Greenl.—Minn. (Fig. 270.)

78. *C. concinna* R. Br. Low Northern Sedge

Caespitose; rootstocks slender, often long; culms 5–20 cm. long, slender, erect or incurved; leaves 2–2.5 mm. wide; terminal spike stami-

nate, 2–3 mm. long, very narrow; lateral spikes two or three, 4–8 mm. long, all crowded at the end of the culm; bracts reduced to sheaths; scales obtuse, reddish-brown, the pistillate with hyaline margins and lighter midrib, ciliate and hairy; perigynia about 3 mm long, obtusely triangular, light-colored, two-ribbed, hairy, the beak dark-colored; achenes triangular.

Dry soil, central Alaska—Newf.—Que.—Mich.—Colo.—B. C. (Fig. 271.)

79. *C. glacialis* Mack. Glacier Sedge

Very densely caespitose; culms 3–15 cm. long, wiry, stiff; leaves 2–4 cm. 1–1.5 mm., flat at base, recurved, triangular and channeled above, stiff; terminal spike staminate, 2–6 x less than 1 mm.; lateral spikes one to three, pistillate, 2–5 mm. long, the entire inflorescence 7–20 mm. long; lowest bract loose, short-tubular, often prolonged into a cusp not over 15 mm. long; scales dark with hyaline margins; perigynia one to five to a spike, about 2.5 mm. long, yellowish-green below, dark above, the beak hyaline-tipped; achenes about 1.75 mm. long.

Alpine-arctic situations, Nome eastward; circumpolar. (Fig. 272) (from Newf.).

80. *C. eburnea* Boott. Bristle-leaved Sedge

Caespitose; rootstocks long, slender; culms 10–35 cm. tall; leaves 0.5 mm. wide; setaceous, involute, firm, often recurved-spreading; terminal spike staminate, 4–8 mm. long; lateral spikes two to four, pistillate, 2–6 x 2 mm., on setaceous peduncles 10–25 mm. long; bracts tubular, truncate; scales whitish with green midrib, often tinged yellowish-brown; perigynia two to six to a spike, 2 x 1 mm., light green or brownish, shining puncticulate, finely nerved, the beak short and hyaline at the orifice; achenes about 2 x 0.75 mm., brown, granular, jointed with the bulbous-thickened base of the style.

Chitina R.—Great Bear L.—Newf.—Va.—Tenn.—Mo.—B. C.

81. *C. rariflora* (Wahl.) J. E. Smith. Loose-flowered Alpine Sedge

Loosely stoloniferous; culms 10–35 cm. tall; leaves 1.5–2.5 mm. wide, the lower very short; terminal spike staminate, 6–15 mm. long, narrow; lateral spikes one or two, 6–15 x 3.5–5 mm.; bracts colored at the base, the lowest usually short-sheathing; scales brownish to blackish, the pistillate darker than the staminate; perigynia two to twelve to a spike, 3–4 mm. long, glaucous-green, two-edged, dark around the orifice; achenes about 2 mm. long, blackish, triangular.

Arctic and central Alaska east; circumpolar. (Fig. 273.)

82. *C. pluriflora* Hult.
C. stygia Auct.

Rhizomes long-stoloniferous, dark or purplish-black; culms 2–5 dm. tall; leaves about equaling the culm, 2–4 mm. wide, flat, roughened toward the attenuate apex; terminal spike staminate, 1–2 cm. long; lateral spikes

one or two, about 13 x 6 mm., on long capillary peduncles; staminate scales reddish-brown with hyaline margins; pistillate scales blackish, acute to abruptly cuspidate; perigynia ten to twenty to a spike, 4–4.5 mm. long, glaucous-green or whitish, later turning brown, papillate, strongly nerved, beakless.

Near the coast, Bering Sea regions—Wash. (Fig. 274.)

83. *C. limosa* L. Shore Sedge

Long-stoloniferous; culms 15–50 cm. tall, rather slender; terminal spike staminate, 1–3 cm. x 2.5 mm.; lateral spikes pistillate or occasionally androgynous, 10–25 x 5–8 mm., drooping on slender peduncles; lowest bract up to 6 cm. long with dark auricles, the upper reduced; scales acute to cuspidate, brownish; perigynia 2.5–4 mm. long, compressed-triangular, glaucous-green, papillate, prominently nerved; achenes about 2.25 mm. long, brown, triangular.

Bering Sea across central Alaska; circumboreal. (Fig. 275.)

84. *C. magellanica* Lam. Bog Sedge
C. paupercula Michx.

Loosely caespitose, with long or short rootstocks; culms 1–4 dm. tall; leaves 2–4 mm. wide, flat with revolute margins; terminal spike staminate or occasionally gynaecandrous, 7–15 mm. long; lateral spikes one to four, usually all pistillate but sometimes gynaecandrous, 4–20 mm. long, drooping on slender peduncles; lowest bract leaf-like, slightly sheathing at the base; scales usually acuminate or cuspidate, brownish, with or without greenish center and tip; perigynia 2.5–3 mm. long, compressed triangular, pale or glaucous-green, papillate, prominently nerved; achene about 2 mm. long.

Seward Peninsula east and south; circumboreal and in S. Am. and the Falkland Islands. (Fig. 276.)

85. *C. laxa,* Wahl. Weak Sedge

Stoloniferous; culms 1–4 dm. tall; slender, weak; leaves 1–2.5 mm. wide, terminal spike staminate, often with a few perigynia, 1–2 cm. long; pistillate spikes one to three, 1–2 cm. long, drooping on capillary peduncles; lowest bract leaf-like with long sheath, scales obtuse, brownish, with rather wide hyaline margins and lighter center; perigynia much as in *C. limosa.*

An old world species reported in America only from Mile 172–174 along the Richardson Highway.

86. *C. livida* (Wahl.) Willd. Livid Sedge

Rootstocks long, slender; culms 1–5 dm. tall; leaves 3 mm. or less wide, glaucous-green, involute, thickened, stiff; terminal spike staminate or with a few perigynia, 7–30 mm. long; pistillate spikes one to three, 10–20 x 5 mm., the lower sometimes subradical; bracts leaf-like, the lower sheathing; scales purplish with hyaline margins and greenish center, the pistillate wider than the staminate; perigynia 2.25–4.5 mm. long, obscurely

triangular, glaucous-green, puncticulate, two-keeled, beakless; achenes triangular, about 2.5 mm. long, brownish-black.

Southwestern Alaska—Labr.—Newf.—N. Jer.—Ida.—Calif. (Fig. 277.)

87. *C. vaginata* Tausch. Sheathed Sedge

C. saltuensis Bailey.

Producing long, horizontal, yellowish-brown stolons; culms 16–80 cm. tall; leaves 1.5–5 mm. wide, flat or channeled toward the base; terminal spike staminate, 1–2 cm. long; lateral spikes two or three, pistillate, 8–20 x 3–5 mm,. the lower ones on long peduncles; bracts with sheaths up to 3 cm. long; scales purplish-brown with hyaline margins and three-nerved light center; perigynia three to twenty to a spike, about 4 mm. long, longer than the scales, yellowish-green or brown, puncticulate, the beak tinged with purple; achenes 2.5–3 mm. long, triangular with concave sides, yellowish.

Alaska Range northward; circumpolar. (Fig. 278.)

88. *C. atrofusca* Schk. Dark-brown Sedge

Caespitose and stoloniferous; culms 1–3 dm. tall, obtusely triangular, usually nodding; leaves clustered at the base, usually less than 1 dm. long but sometimes longer, 2–4 mm wide; terminal spike gynaecandrous, the lateral spikes two to three, 8–18 mm. long, drooping on slender peduncles; lowest bract long-sheathing; scales black with somewhat lighter margins and midribs; perigynia 4–5 mm. long, dark with lighter base and hyaline at the tip of the beak, granular, two-ribbed, slightly serrulate above; achenes conspicuously stipitate and apiculate.

Bering Sea eastward; circumpolar. (Fig. 279.)

89. *C. misandra* R. Br. Short-leaved Sedge

Caespitose; culms slender, 1–3 dm. tall; leaves 1.5–3 mm. wide, canaliculate below, thickish, stiff, long-attenuate; terminal spike gynaecandrous, drooping; lateral spikes two or three, pistillate, 7–20 x 4–6 mm.; lowest bract long-sheathing, the sheath tight, tinged purplish, the blade short; perigynia 4–6 mm. long, 1 mm. wide, flattened-triangular, dark above, light-colored below, ciliate-serrulate on the margin above, two-edged, the beak with a hyaline tip; achenes about 2 x 0.75 mm., brownish.

Arctic, Bering Sea and Pacific coastal districts. Distribution interrupted circumpolar. (Fig. 280.)

90. *C. capillaris* L. Hair-like Sedge

Caespitose; culms 1–6 dm. long, very slender, erect, spreading or decumbent; leaves 0.75–2.5 mm. wide; terminal spike staminate, 4–8 mm. long; lateral spikes two or three, pistillate, 5–15 mm. long, on slender drooping peduncles; bracts sheathing, tubular; scales ovate, hyaline-margined, shorter than the perigynia; perigynia 2.5–3 mm. long, obtusely triangular, somewhat inflated, greenish-brown, two-ribbed, the beak hyaline-tipped; achenes 1.5 mm. long, triangular.

Throughout most of Alaska—Greenl.—Maine—N. Mex.—Nev.—B. C. (Fig. 281.)

91. *C. krausei* Boeck. Krause Sedge

C. capillaris var. *nana* (Cham.) Kuk.

Caespitose; culms 3–30 cm. tall; leaves nearly as long as the culms, sometimes longer; terminal spike gynaecandrous. Resembles *C. capillaris* but is of much lower growth, the terminal spike is gynaecandrous, the perigynia have shorter beaks which are finely spinulose on the margins.

Central and southeastern Alaska—Yukon. Range probably more extensive.

92. *C. williamsii* Britt. Williams Sedge

Caespitose; culms 3–30 cm. tall, slender; leaves 2–8 cm. long, 0.25–0.75 mm. wide, canaliculate, minutely serrulate toward the base; terminal spike staminate 2–6 x 0.5–1 mm.; lateral spikes three to five, pistillate, 4–10 x 2.5 mm.; lowest bract tubular-sheathing; scales obtuse or the staminate mucronate; perigynia three to nine to a spike, 2.5–3.5 mm. long, narrow, greenish, puncticulate, the beak with hyaline orifice; achenes 1.5 x 0.5 mm.

Bering Sea region—Labr.

93. *C. flava* L. Yellow Sedge

Caespitose; culms 1–8 dm. tall, stiff; leaves 3–5 mm. wide, flat or canaliculate at the base, thickish; terminal spike staminate or with a few perigynia, 5–20 mm. long; lateral spikes two to five, pistillate or sometimes androgynous, 7–18 x 10 mm.; bracts leaf-like, the lowest with sheath 2–20 mm. long; scales reddish-brown with hyaline margins and lighter center; perigynia 4.5–6 x 1.25–2 mm., spreading or deflexed, yellowish-green or yellow, puncticulate, ribbed; beak 2–3 mm. long, serrulate, the teeth of the bidentate beak tinged with red; achenes 1.5 x 1 mm.

Yakutat Bay—Hudson Bay—Labr. .

94. *C. viridula* Michx. Green Sedge

C. oederi Retz. var. *viridula* (Michx.) Kuk.

Caespitose; culms 6–30 cm. tall, stiff; leaves 1–3 mm. wide, canaliculate, thickish; terminal spike staminate, 3–15 mm. long; lateral spikes two to six, pistillate, 5–10 x 4–7 mm.; bracts leaf-like, the lowest with sheath 4–18 mm. long; scales shaded brownish; perigynia fifteen to thirty to a spike, 2–3 mm. long, spreading, yellowish-green, puncticulate, ribbed, the beak scarcely half as long as the body; achenes 1.25 x 1 mm., blackish.

Southeastern Alaska—Sask.—Utah—N. Mex.—Calif. Also in eastern America and eastern Asia. (Fig. 282.)

95. *C. rostrata* Stokes. Beaked Sedge

Caespitose and long-stoloniferous; culms 3–12 dm. tall, tinged red at the base; leaves 2–12 mm. wide, flat above and with revolute margins, septate-nodulose; upper two to five spikes staminate, 1–7 cm. long, narrow.

lower two to five spikes pistillate or some of them androgynous, 1–8 cm. x 4–8 mm., bracts leaf-like; scales brownish with hyaline margins and lighter center; perigynia 4–8 mm. long, inflated, yellowish-green to brown, puncticulate, strongly nerved, the beak bidentate; achenes 2 mm. long, yellowish-brown.

All of our area except the high arctic; circumboreal. (Fig. 283.)

96. *C. rotundata* Wahl. Round-fruited Sedge
C. melozitensis Porsild.

Loosely caespitose and with long stolons; culms 15–45 cm. tall; leaves 1–3 mm. wide, involute; terminal spike staminate, 10–25 mm. long, often with one or two smaller ones at the base; lateral spikes one or two, pistillate, 8–25 x 6–9 mm.; lowest bract leaf-like, 1–3 cm. long; staminate scales brown, the pistillate purplish-black, both with hyaline apex and lighter midrib; perigynia 3–3.5 mm. long, inflated, straw-colored, tinged brownish, puncticulate; achenes 2.25 mm. long, grayish-brown.

Bering Sea and Alaska Range north and east; circumpolar. (Fig. 284.)

97. *C. rhyncophysa* C. A. Mey.
C. laevirostris (Blytt) Fr.

Caespitose and with long stolons; culms 4–10 dm. tall, stout; leaves 6–15 mm. wide, flat above, channeled below, firm, strongly septate-nodulose; upper two to four spikes staminate, 2–6 cm. long, lower two to five spikes pistillate, 25–75 x 9–12 mm.; lower bracts leaf-like, sheathless or nearly so; staminate scales obtusish, pistillate scales acute or acuminate, both tinged reddish-brown with hyaline margins and lighter center; perigynia 4.5–7 mm. long, inflated, greenish straw-color, coarsely nerved, long-beaked; achenes 2 x 1.5 mm., triangular with concave sides below.

Matanuska—Yukon—Mack.—Eurasia. (Fig. 285.)

98. *C. physocarpa* Presl.

Rootstocks long, slender; culms 2–8 dm. tall; leaves 1.5–5 mm. wide; terminal spike staminate, 2–4 cm. long, often with one or two shorter ones at the base; lateral spikes one to three, pistillate, 15–35 x 6–12 mm., spreading or drooping on slender peduncles; lowest bract leaf-like; scales purplish-black with lighter midrib and prominently hyaline tips; perigynia 4–5 mm. long, dull grayish-yellow, usually dark tinged above; achenes 1.5–2 mm. long, lenticular, yellow, continuous with the bent style. This species forms hybrids with *C. rostrata* (*C. utriculata* Cov. & Wight).

Common in our area except the Arctic and extends eastward to Mack. and south to Colo. & Utah. Also in eastern Asia. (Fig. 286.)

99. *C. membranacea* Hook. Fragile Sedge
C. membranopacta Bailey.

Caespitose and stoloniferous, culms 15–50 cm. tall, stiff; leaves 3–5 mm. wide, flat, often with revolute margins; terminal spike staminate, 10–25 mm. long, sometimes with one or two smaller ones at the base; pistillate spikes one to three, sessile to short-peduncled, 12–30 mm. long, 7–9 mm.

thick; bracts leaf-like, the upper reduced; scales dark, often blackish, usually with hyaline margins and lighter center; perigynia 3.5–4.5 mm. long, inflated, fragile, dark-tinged above, the beak bidentate; achenes about 1.5 mm. long.

Throughout most of our area—Ellsmereland—Greenl.—Ungava. Also in Asia. (Fig. 287.)

100. *C. atherodes* Spreng. Awned Sedge

Loosely caespitose and stoloniferous; culms 5–15 dm. tall; leaves 3–12 mm. wide, flat, thin, septate-nodulose, sparsely hairy beneath toward the base, the sheaths soft-hairy; upper two to six spikes staminate, 4–10 cm. long; lower two to four spikes pistillate or androgynous, 5–12 cm. x 8–15 mm.; bracts leaf-like, the lowest sheathing; scales rough-aristate, ciliate; perigynia 7–12 x 2 mm., inflated, yellowish-green or brownish, strongly ribbed, the beak bidentate with long, spreading teeth; achenes 2.5 x 1.25 mm., triangular, yellowish-brown.

Yukon—Mack.—St. Lawrence R.—N. Jer.—Mo.—Utah.—Ore.

In addition to the species here described several other species of *Carex* have been reported from our area but their occurrence needs confirmation.

8. ARACEAE (Arum Family)

Perennials with basal, reticulate-veined, petioled leaves and the flowers in a dense, fleshy spike borne on a spadix subtended or enclosed by a large foliaceous or colored bract (spathe). Perianth of scale-like parts or none; flowers in ours perfect; stamens four to ten; fruit a berry or utricle.

Flowers with a perianth, spathe yellow 1. *Lysichitum*
Flowers without a perianth, spathe white 2. *Calla*

1. LYSICHITUM Schott.

Swamp plants with short, thick rootstocks, large fleshy roots and large, fleshy leaves, with the petioles sheathing at the base; spadix at first enveloped by the spathe, later exerted; perianth of four segments; stamens four; filaments flat; anthers two-celled; ovary conical, two-celled, two-ovuled; stigma depressed; fruit fleshy. Also spelled Lysichiton. (Greek, base and tunic, referring to the spathe.)

L. americanum Hult. & St. J. Skunk Cabbage

Large tropical-looking swamp plants with elliptic or oblong-lanceolate leaves, a large blade having actually measured more than 13 dm. long and 7 dm. wide; spathe yellow, oblong-lanceolate, acute, the open part 1–3 dm. long; spadix 7–12 cm. long in flower, elongating in fruit, on a stout, fleshy peduncle.

Pacific coastal district of Alaska—Mont.—Calif. (Fig. 288.)

2. CALLA L.

A bog or aquatic herb; rootstocks creeping, acrid; leaves broadly ovate-cordate; spathe white, ovate-lanceolate, acuminate with a long point;

spadix cylindric, densely covered with flowers; stamens about six; anthers with two divaricate sacs; ovary ovoid, one-celled; ovules six to nine; berries depressed-obconic. (Ancient name.)

C. palustris L. Water Arum

Petioles 1–3 dm. long; leaf-blades 6–12 cm. long, 4–9 cm. wide, cuspidate at the apex, cordate at the base; scapes as long as the petioles; spathe 4–7 cm. long, the mucronation often 6–8 mm. long; fruiting spadix 2–3 cm. long and half as wide.

Shallow water, southwestern and central Alaska eastward; circumboreal. (Fig. 289.)

9. LEMNACEAE L. (Duckweed Family)

Minute stemless and leafless perennial plants with thallus-like body, floating on fresh water; roots one or more from the lower surface; inflorescence of one or more monoecious flwers borne on the edge of the upper surface, staminate flower of a single stamen with two to four pollen-sacs; pistillate flower of a single flask-like pistil; fruit a one to six seeded utricle. The simplest and smallest of flowering plants, propagating mostly by budding.

LEMNA L.

Fronds disk-like or oval; rootlet solitary, without fibrovascular tissues; anthers dehiscent transversely. Not often found in flower. (Greek, in allusion to the swamp habitat.)

Fronds long-tailed, mostly submerged 1. *L. trisulca*
Fronds short-stalked or sessile, floating 2. *L. minor*

1. *L. trisulca* L. Ivy-leaved Duckweed

Fronds usually submerged with several generations attached to each other, oblong, or oblong-lanceolate, 6–10 mm. long, 2–3 mm. wide, obscurely three-nerved and denticulate at the apex, often without rootlets.

Cook Inlet district, circumboreal. (Fig. 290.)

2. *L. minor* L. Lesser Duckweed

Fronds 2–4 mm. wide, rounded to obovate-oblong, symmetrical, green or rarely reddish or purplish-tinged, obscurely three-nerved, and often a row of papillae on the midrib; fruit symmetrical, subturbinate; seed deeply and unevenly 12–15-ribbed. (Fig. 291.)

10. JUNCACEAE (Rush Family)

Annual or mostly perennial grass-like herbs; flowers perfect; regular inconspicuous, sepals and petals each three, similar and scale-like; stamens six or three, rarely four or five; anthers introrse; pistil of three united carpels; ovary one or three-celled; stigmas three, filiform; capsule loculicidal; seed three to many, usually reticulated or ribbed and often tailed.

Leaf-sheaths open, capsule many-seeded 1. *Juncus*
Leaf-sheaths closed, seeds three 2. *Luzula*

1. JUNCUS (Tourn.) L.

Mostly glabrous perennial swamp plants, with or without leaves, leaf-sheaths with free margins, the blades terete, gladiate, grass-like or channeled; flowers subtended by a bract, sometimes also by two bractlets; capsules many-seeded. Our illustrations show the mature capsule surrounded by the perianth, seed, and the bractlets when present. (Latin, *jungo*, to bind, in allusion to the use of these plants for withes.)

1A. Inflorescence appearing lateral due to the prolongation of the lower bract.
- 1B. Bract about as long as the stem 1. *J. filiformis*
- 2B. Bract shorter than the stem.
 - 1C. Involucral bract short, 0.5–3 cm. long..... 2. *J. drummondii*
 - 2C. Involucral bract longer.
 - 1D. Stems tufted 3. *J. effusus*
 - 2D. Stems in rows from horizontal rhizomes.
 - 1E. Involucral bracts 2–5 cm. long..... 4. *J. arcticus*
 - 2E. Involucral bracts longer.
 - 1F. Anthers about 2 mm. long 6. *J. ater*
 - 2F. Anthers shorter 5. *J. balticus*

2A. Inflorescence appearing terminal.
- 1B. Leaves gladiate 7. *J. ensifolius*
- 2B. Leaves terete, convolute, or channeled.
 - 1C. Flowers borne separately.
 - 1D. Low annual 8. *J. bufonius*
 - 2D. Tall perennial 9. *J. macer*
 - 2C. Flowers in one or more compact heads.
 - 1D. Heads normally one.
 - 1E. Heads many-flowered 13. *J. mertensianus*
 - 2E. Heads 1–5 flowered.
 - 1F. Stem with leaf in its middle part 10. *J. stygius*
 - 2F. Stem with only basal leaves.
 - 1G. Involucral bract overtopping the head 11. *J. biglumis*
 - 2G. Involucral bract shorter 12. *J. triglumis*
 - 2D. Heads normally more than one.
 - 1E. Leaves septate, terete.
 - 1F. Heads globose 16. *J. nodosus*
 - 2F. Heads conical with erect or ascending flowers.
 - 1G. Perianth 3.5–5 mm. long.....14. *J. oreganus*
 - 2G. Perianth 2–3 mm. long 15. *J. alpinus*
 - 2E. Leaves not septate.
 - 1F. Capsule truncate or depressed at the apex 17. *J. falcatus*
 - 2F. Capsule rounded or acute at the apex.
 - 1G. Capsule acutish, pale, about double the length of the perianth 19. *J. leucochlamys*

2G. Capsule obtusish, dark, only slightly longer than the perianth 18. *J. castaneus*

1. *J. filiformis* L. Thread Rush

Stems tufted from creeping rootstocks, 1–6 dm. tall, about 1 mm. thick; basal leaf-blades filiform rudiments; lower leaf of inflorescence erect and often longer than the stem; inflorescence several-flowered, spreading; perianth about 3 mm. long, the segments lanceolate, acute; stamens six, about half as long as the perianth; capsule green, barely pointed, as long as or shorter than the perianth; seeds obliquely oblong, pointed at one or both ends.

St. George Isl.—southeastern and central Alaska; circumboreal. (Fig. 292.)

2. *J. drummondii* E. Mey. Drummond Rush

Stems densely tufted, 1–4 dm. tall, from matted rootstocks; leaf-sheaths bladeless or with mere rudiments; inflorescence one to five, but mostly three-flowered; lower bract 10–25 mm. long; flowers with a pair of brown bractlets at the base; perianth about 6 mm. long, the segments lanceolate, acute or acuminate with broad brown margins; stamens six, about half the length of the perianth; seeds caudate at both ends.

Aleutians—Alta.—N. Mex.—Calif. (Fig. 293.)

3. *J. effusus* L. Bog Rush

Stems tufted, 5–14 dm. tall; basal leaf-blades reduced to short filiform rudiments; inflorescence many-flowered, usually dense and congested, 2–5 cm. long; lowest bract of the inflorescence 5–25 cm. long, perianth 2–3 mm. long, the segments lanceolate, acuminate; stamens three; capsule obovoid, about as long as the perianth; seed with short points, two or three times as long as broad.

Along the coast, southeastern Alaska—Calif. (Fig. 294.)

4. *J. arcticus* Willd. Arctic Rush

Stems arising at close intervals from creeping rootstocks and somewhat caespitose, 12–35 cm. tall; sheaths leafless or with a small mucronation; inflorescence three to ten flowered; lowest bract 4–20 cm. long; perianth 4–5 mm. long, the outer segments somewhat longer than the inner and darker brown, all with light center; stamens shorter than the perianth, the anther about the same length as the filament; capsule dark and shining, about the same length as the perianth; seed somewhat irregular in shape, about 0.5 mm. long. Most of our forms belong to the ssp. *alaskanus* Hult. with more lax inflorescence and more acute inner segments of the perianth.

Arctic and Bering Sea coasts eastward; circumpolar. (Fig. 295.)

5. *J. balticus* Willd. Baltic Rush

Stems arising from creeping rootstocks, 2–8 dm. tall; sheaths leafless or with a slender mucronation; main floral bract 6–20 cm. long; per-

ianth 4–5 mm. long, the segments lanceolate, the outer more acute than the inner, purplish-brown; anther as long as or longer than the filament; capsule brown and shining, narrowly ovoid, conspicuously mucronate, fully as long as the perianth; seed striate. The typical form occurs in southeastern Alaska but is not so common as the ssp. *sitchensis* (Engelm.) Hult., with anthers about as long as the filaments and the inner perianth segments more narrowly scarious-margined, which occurs over most of our area south of the Arctic Circle.

Whole species circumboreal; the ssp. in eastern Asia and Alaska. (Fig. 296.)

6. *J. ater* Rydb. Mountain Rush

Stems slender, about 2 mm. thick; flowers 5–25; anthers about 2 mm. long on very short filaments. Resembles *J. balticus* and its variety but the stem is more slender, the inflorescence is more lax, the lower floral bract averages shorter, the inner perianth segments are more acute with narrower hyaline margins and the anthers are longer.

Central Alaska—Mont.—Nebr.—N. Mex.—Calif.

7. *J. ensifolius* Wiks. Dagger-leaved Rush

Stems flattened, leafy, erect, 2–6 dm. tall, arising from creeping rootstocks; leaves flattened laterally and equitant, 7–25 cm. long, 3–6 mm. wide; heads one to seven, very dense; perianth dark brown, the segments lanceolate, acuminate, about 3 mm. long; stamens three, two-thirds as long as the perianth; capsule dark brown, obtuse below the mucronation, slightly exceeding the perianth.

Eastern Asia—Aleutian Islands—Alta.—Utah—Calif. (Fig. 297.)

8. *J. bufonius* L. Toad Rush

Low, profusely branched annual 5–25 cm. tall; leafy below, the leaves narrow and involute, the lower up to 5 cm. long, the upper short; flowers greenish, inserted singly on the branches and in the axils of the leaves; perianth 4–6 mm. long, the inner segments shorter and less attenuate than the outer; capsule shorter than the perianth; seeds broadly oblong but variable.

Nearly cosmopolitan but may be introduced in our area. (Fig. 298.)

9. *J. macer* S. F. Gray. Slender Rush

Stems wiry, tufted, 2–6 dm. tall; leaves from one-half to nearly as long as the stem, flat; bracts two or three, leaf-like, at least one of them longer than the open inflorescence; flowers greenish, aggregated at the top of, or scattered along the branches of the panicle; perianth segments lanceolate with scarious margins, acute, 3–4.5 mm. long; capsule broadly ovoid, obscurely triangular.

Introduced in southeastern Alaska. Has been confused with *J. tenuis* Willd. (Fig. 299.)

10. *J. stygius* L. ssp. *americanus* (Buch.) Hult. Moor Rush

Stems 7–15 cm. tall, erect, one- to three-leaved below; leaves erect or ascending, the sheaths nerved and auriculate; heads usually one, some-

times more, one to four flowered; lowest bract usually exceeding the flowers; perianth 3–5 mm. long, the parts about equal; capsule 6–8 mm. long, spindle-shaped, pale brown, acute, mucronate, few-seeded; seeds with a thick coat forming thick tails, the total length 2.5–3 mm.

Central Alaska—Labr.—Newf.—Gt. Lakes region. The typical form in Europe and western Asia. (Fig. 300.)

11. *J. biglumis* L. Two-flowered Rush

Stems one to few, from branched rootstocks, erect, 25–200 mm. tall; leaves one to five, all basal; longest bract of the inflorescence foliose, 5–25 mm. long; flowers one to four, usually two, perianth 3–4 mm. long, dark brown, the segments about equal, obtuse; stamens equaling the perianth; capsule exceeding the perianth, retuse at apex with three-keeled shoulders; seed 1 mm. long or more, brown with broad white tails.

Western and central Alaska—Ellsmereland—Greenl.—Hudson Bay—Labr.—arctic Eurasia. (Fig. 301.)

12. *J. triglumis* L. Three-flowered Rush

J. albescens (Lge.) Fern.

Stems tufted, 5–15 cm. tall; leaves one to five, all basal with clasping, auriculate sheaths and narrow terete blades 1–7 cm. long; inflorescence a cluster of one to five, usually three, flowers; the lowest two or three bracts divergent, usually brown and membranous, perianth about 4 mm. long; stamens nearly as long as the perianth; capsule about equaling the perianth, mucronate; seed less than 2 mm. long including the tails.

Bering Strait east and south; circumboreal. (Fig. 302.)

13. *J. mertensianus* Bong. Mertens Rush

Stems tufted, erect, 8–30 cm. tall, 1–1.5 mm. thick; leaves two or three on the stems, occasionally overtopping the stem; inflorescence a dense head 8–15 mm. broad, capitate, the lower bract usually longer than the head; perianth nearly black, about 4 mm. long, stamens nearly equaling the perianth, the anthers much shorter than the filaments; capsule scarcely as long as the perianth.

Alpine meadows, Japan, Aleutian Islands and central Alaska—Colo.—Calif. (Fig. 303.)

14. *J. oreganus* S. Wats. Oregon Rush

Stems tufted from slender, matted rootstocks, 1–2 dm. tall; stem leaves two to four, the sheaths with conspicuous hyaline margins and auricles; heads usually two to four, small, few- several-flowered; perianth segments nearly equal, narrowly lanceolate, acute, brown; stamens half as long as the perianth, the filaments longer than the anthers; capsule often twice as long as the perianth, acute mucronate, dark brown; seeds ribbed and cross-lined.

Bogs near the coast, Kodiak Island—Ore.

15. *J. alpinus* Vill. Richardson Rush

J. richardsonianus Schult.

Stems erect, 15–50 cm. tall from creeping rootstocks; one or two-leaved, leaf-blades terete or slightly compressed, septate; flowers in pan-

icled heads of two to twelve; perianth 2–2.5 mm. long, the inner segments shorter than the outer; capsule ovoid-oblong, longer than the perianth, straw-colored or light brown; seed apiculate, acute or acuminate at the base. Except in the Pacific coast and the Aleutian Islands districts our forms belong to the ssp. *nodulosus* (Wahl.) Lindm. (*J. nodulosis* Wahl.) which has some of the flowers peduncled in the loose heads, whereas in the type all flowers are sessile.

Central Alaska southward; circumboreal. (Fig. 304.)

16. *J. nodosus* L. Knotted Rush

Stems 15–60 cm. tall, arising from thickenings of slender rootstocks; leaves erect, conspicuously septate-nodulose; inflorescence of one to thirty heads 7–12 mm. in diameter, six to twenty flowered; perianth 3–4 mm. long, the inner segments longer than the outer; capsule acutely triangular, long-pointed, usually longer than the perianth; seed acute below, apiculate above, reticulate.

Manly Hot Springs and southeastern Alaska—Newf.—Va.—N. Mex. —Nev. (Fig. 305.)

17. *J. falcatus* E. Mey. ssp. *sitchensis* (Buch.) Hult. Sickleleaved Rush
J. falcatus var. *sitchensis* Buch.

Stems 8–30 cm. tall from slender creeping rootstocks; basal leaves grass-like, from two-thirds as long to longer than the stems, 1.5–3 mm. wide; stem leaves one or two; heads one to six, the lowest bract foliaceous; perianth about 4 mm. long, minutely roughened, the segments about equal, the outer minutely mucronate, brown or with green midrib; capsule slightly retuse, as long as or longer than the perianth; seeds reticulate, with a light-colored coat.

Eastern Asia—Aleutian Islands—southeastern Alaska. The type form from B. C.—Calif., Japan and Tasmania. (Fig. 306.)

18. *J. castaneus* J. E. Smith. Chestnut Rush

Stoloniferous; stems erect, 1–5 dm. tall, more or less leafy; leaves 4–12 cm. long, tapering from an involute tubular base to a slender channeled apex; heads one to four, three- to twelve-flowered; perianth brown, about 5 mm. long, the segments lanceolate, acute; capsule dark brown, paler toward the base, longer than the perianth; seeds with long, light brown tails.

Found in most of our area; circumboreal. (Fig. 307.)

19. *J. leucochlamys* Zing. & Kretch.

Resembles *J. castaneus* but is taller, has from two to twelve heads, the capsules are longer and more acute, being about twice as long as the perianth; the perianth and capsule are much lighter in color, being a pale brown.

Eastern Asia, known in America only from Matanuska.

2. LUZULA DC.

Glabrous or sparingly pubescent plants with leaf-bearing stems, the leaves grass-like with closed sheaths; inflorescence umbel-like, capitate,

or spike-like; flowers always subtended by usually dentate or lacerate bractlets; stamens six; ovary one-celled, developing into a three-seeded capsule. (Latin, *lux*, light, suggested by leaves of a species shining with dew. *Juncoides* (Dill.) Adans.)

1A. Inflorescence umbel-like 1. *L. rufescens*
2A. Inflorescence an open panicle with usually solitary flowers.
1B. Bractlets lacerate and abundantly ciliate..... 2. *L. wahlenbergii*
2B. Bractlets less lacerate and glabrous or with a single cilium 3. *L. parviflora*
3A. Inflorescence of one to several spike-like or head-like glomerules of flowers.
1B. Flowers in a dense, drooping, spike-like panicle..4. *L. spicata*
2B. Flowers in glomerules or sometimes in an erect spike in number six.
1C. Leaves involute or channeled, with purplish bases.
1D. Inflorescence widely branched with curved branches 5. *L. arcuata*
2D. Inflorescence spike-like or sparsely branched with straight branches 6. *L. confusa*
2C. Leaves flat with brownish bases.
1D. Bract shorter than the inflorescence..... 7. *L. nivalis*
2D. Bract longer than the inflorescence..... 8. *L. multiflora*

1. *L. rufescens* Fisch. Hairy Wood-Rush

Stoloniferous and somewhat caespitose; stems slender, 1–3 dm. tall; leaves mostly basal, 1–3 mm. wide, slightly webbed when young; inflorescence umbellate, some of the pedicels often reflexed, rarely bearing more than one flower; perianth 2–3 mm. long; capsule acute, longer than the perianth; seed with a conspicuous irregular caruncle. Reports of *Luzula* (or *Juncoides*) *carolinae*, *japonica*, *pilosa* or *saltuensis* from Alaska all refer to this species.

Eastern Asia extending to east central Alaska. (Fig. 308.)

2. *L. wahlenbergii* Rupr. Wahlenberg Wood-Rush

Stems caespitose, erect, 10–35 cm. tall; leaves mostly basal, usually not more than 3 mm. wide; inflorescence diffuse, the capillary branches curved; flowers solitary but often two to four approximate; bractlets lacerate or ciliate; perianth 2.25–3 mm. long, the segments acute, brown with hyaline tips; capsule about equaling the perianth, ovoid; seed about 1.5 mm. long, brown, attached to the placentas by white fibers. Along the southern Alaska coast this species approaches *L. parviflora*.

Seward Peninsula east and south; circumboreal. (Fig. 309.)

3. *L. parviflora* (Ehrh.) Desv. Small-flowered Wood-Rush
J. parviflorum (Ehrh.) Cov.

Stems erect, terete, 3–6 dm. tall; leaves 5–15 cm. long, 4–12 mm. wide, glabrous except for a few hairs at the mouth of the sheath; inflorescence a nodding, compound panicle, the flowers usually borne singly on slender

pedicels; perianth 2–2.5 mm. long, the segments lanceolate, acute, green or brown; capsule ovoid, dark, slightly exceeding the perianth; seeds ellipsoid, brown, with cottony fibers at lower ends. The ssp. *divaricata* (S. Wats.) Hult., has larger, more spreading panicle with lighter colored flowers. This and the preceding species sometimes form hybrids.

Kotzebue Sound—Baffinland south; circumpolar. (Fig. 310.)

4. *L. spicata* (L.) DC. Spiked Wood-Rush
J. spicatum (L.) Kuntze.

Stems tufted, erect, 1–3 dm. tall; leaves 4–12 cm. long, 1–3 mm. wide, with sharp apex and sparingly webby; inflorescence spike-like, usually nodding; bractlets ovate-lanceolate, acuminate, more or less webby, often equaling the perianth; perianth dark brown with lighter margins; capsule acute, shorter than the perianth; seeds brown with light base, about 1 mm. long.

Central Alaska southward; circumpolar. (Fig. 311.)

5. *L. arcuata* Wahl. Alpine Wood-Rush
J. arcuatum (Wahl.) Kuntze.

Caespitose and short-stoloniferous; stems 8–20 cm. tall, slender; leaves 1–3 mm. wide, usually some of them curved, canaliculate, the apex subulate; inflorescence paniculate, the branches curved and spreading; perianth brown, 2–2.5 mm. long, the parts about equal; capsule slightly shorter than the perianth; apiculate; seed attached with a tuft of white fibers.

Seward Peninsula—Wash., and in Eurasia. (Fig. 312.)

6. *L. hyperborea* R. Br. Northern Wood-Rush
L. confusa Lindeb.
J. hyperborium (R. Br.) Sheldon.

Stems tufted, erect, 1–3 dm. tall; leaves erect, 1–3 mm. wide, sparingly ciliate at the mouth of the sheath, sharp-pointed; inflorescence of a single head or two- or three-branched, the branches erect or curved; lowest bract short-foliose, the upper bracts and the bractlets fimbriate; perianth 2–2.5 mm. long, the parts brown, paler above; capsule somewhat shorter than the perianth, ovoid.

Arctic and central Alaska; circumpolar. (Fig. 313.)

7. *L. nivalis* (Laest.) Beurl. Snow Wood-Rush
J. arcticum Am. auct.

Stems tufted, 5–10 cm. tall, erect; leaves 2–4 mm. wide, usually less than 8 cm. long; inflorescence an ovate, spike-like cluster about 1 cm. long; perianth 2 mm. long or less. Known from Little Diomede Island and southeastern Alaska. The common form is the var. *latifolia* Kjellm. Stems 8–25 cm. tall; leaves 3–5 mm. wide; inflorescence with one to three slender, erect or curved branches; bractlets with hyaline, more or less laciniate margins, one-half to two-twirds as long as the perianth; perianth 2.5–3 mm. long, its parts equal; capsule as long as the perianth or shorter; seed brown with dark tip and light base.

A circumpolar species. (Fig. 314.)

8. *L. multiflora* (Retz.) Lej. Many-flowered Wood-Rush
L. campestris Am. auct.
J. campestris Am. auct.

Stems tufted, 1–5 dm. tall; leaves ciliate on the margins, webbed at the mouth of the sheath; inflorescence of four to ten, eight to sixteen-flowered heads, the branches erect or ascending, sometimes congested; lowest bract foliose, longer than the inflorescence, bractlets often entirely hyaline; perianth 2.5–3.5 mm. long, brown with hyaline margins; capsule somewhat shorter than the perianth; seed with white caruncle at the base. Var. *frigida* (Buch.) G. Sam. Stems 10–15 cm. tall; leaves 2–4 mm. wide, inflorescence dense. Var. *kobayasii* (Satake) G. Sam. Leaves up to 1 cm. wide. Ssp. *comosa* (E. Mey.) Hult. High growing; bract large; bractlets strongly ciliate; perianth yellowish.

A variable, circumboreal species. (Fig. 315.)

11. MELANTHACEAE (Bunch-flower Family)

Leafy-stemmed or scapose perennials with elongated or bulb-like rootstocks; leaves alternate or basal; flowers perfect, dioecious, or polygamous in racemes or panicles; sepals and petals distinct or nearly so; stamens six, often partly adnate to the base of the sepals and petals; anthers versatile; pistil composed of three united carpels; ovary three-celled; styles three; fruit a septicidal capsule. By many authors this group is considered to be only a tribe or subfamily of *Liliaceae*.

1A. Plants with bulbs 3. *Zygadenus*
2A. Plants with rootstocks.
1B. Leaves narrow 1. *Tofieldia*
2B. Leaves broad 2. *Veratrum*

1. TOFIELDIA Huds.

Rootstocks short with numerous fibrous roots; leaves two-ranked, linear or equitant; flowers small, in a terminal spike-like raceme, subtended by a small involucre of three, more or less, united bractlets below the perianth; sepals and petals nearly equal, persistent, glandless; capsule septicidal to the base; seeds numerous. (Tofield was a botanist of Yorkshire, England.)

1A. Stems viscid-pubescent above 1. *T. occidentalis*
2A. Stems glabrous, scapiform.
1B. Flowers greenish 2. *T. pusilla*
2B. Flowers purplish 3. *T. coccinea*

1. *T. occidentalis* S. Wats. Western Tofieldia
T. intermedia Rydb.

Stems 15–20 cm. tall, viscid above with black, stalked glands; leaves 5–25 cm. long, 2–6 mm. wide; racemes 15–50 mm. long, rather dense, the pedicels usually three together; flowers greenish-yellow, the sepals ovate, 4–5 mm. long, the petals narrower and slightly longer; capsule ovoid, 5–7 mm. long. Most of the Alaskan material belongs to the form described as

T. intermedia but the characters on which that form is based are not constant.

Southern Alaska—Sask.—Calif. (Fig. 316.)

2. *T. pusilla* (Michx.) Pers. Scotch Asphodel, False Asphodel
T. palustris Am. auct.

Stems tufted, 6–18 cm. tall; leaves 2–10 cm. long, gladiate; raceme 6–25 mm. long, usually rather dense; flowers yellowish-green on short pedicels; sepals and petals obovate, much shorter than the oblong-globose, beaked capsule.

A circumpolar species occurring in most of our area. (Fig. 317.)

3. *T. coccinea* Richards. Northern Asphodel
T. nutans Willd.

Stems tufted, 4–10 cm. tall; basal leaves gladiate, 2–6 cm. long, 2–4 mm. wide; raceme short and dense; flowers short-pedicelled or nearly sessile, tinged with purple; petals and sepals obovate, nearly as long as the depressed globose, minutely beaked, dark purple capsule.

A circumpolar species occurring almost throughout our area. (Fig. 318.)

2. VERATRUM (Tourn.) L.

Tall, stout, poisonous perennials; rootstocks stout; leaves broad, strongly veined and plaited; flowers polygamous, in large panicles; sepals and petals each three, nearly equal; stamens six; anthers cordate; fruit a many-seeded, slightly inferior capsule; seeds flat, winged. (Ancient name of the Hellebore.)

Flowers yellowish-green 1. *V. eschscholtzii*
Flowers whitish inside 2. *V. album*

1. *V. eschscholtzii* A. Gray. American White Hellebore
V. viride Ait. in part.

Stems 10–25 dm. tall; leaves broadly round-oval to ovate-lanceolate, narrower toward the inflorescence, glabrous above, pubescent, often densely so, below, up to 3 dm. long and 2 dm. wide, the base sheathing the stem; inflorescence a large panicle with drooping branches; sepals and petals oblanceolate, greenish, 8–10 mm. long, about twice as long as the stamens; capsule 10–12 mm. long. May be only a variety of *V. viride*.

Bering Sea—Mont.—Calif. (Fig. 319.)

2. *V. album* L. ssp. *oxysepalum* (Turcz.) Hult. European White Hellebore

Resembling the preceding species but not so tall and with an upright, spike-like inflorescence, the branches short and ascending; flowers whitish, on very short pedicels; lower surface of leaves glabrous or pubescent on the veins only. An old world species found on Attu Island and on Seward Peninsula extending toward central Alaska.

3. ZYGADENUS Michx.

Glabrous or obscurely scabrous perennials; bulbs membranous-coated; leaves linear, mainly basal; flowers in terminal racemes, perfect or

polygamous; petals and sepals withering-persistent, sometimes adnate to the base of the ovary, bearing one or two glands just above the narrowed base; anthers cordate or peltate, one-celled; capsule three-lobed, three-celled; seed angled. (Greek, yoke and gland, referring to the pair of glands in some species.)

Z. elegans Pursh. Glaucus Zygadenus
Z. chloranthus Richards
Anticlea elegans (Pursh.) Rydb.

Stems glabrous, 3–6 dm. tall; basal leaves 1–3 dm. long, 5–15 mm. wide, slightly keeled, the few stem leaves shorter; bracts lanceolate, rather large, often purplish; flowers greenish-white; sepals and petals obovate or oval, obtuse, nearly 1 cm. long; gland obcordate; capsule ovoid, about 15 mm. long.

Most of Alaska—Gt. Bear Lake—Sask.—Minn.—Wash. (Fig. 320.)

12. LILIACEAE (Lily Family)

Caulescent or scapose perennials, our species from bulbs; leaves various; flowers solitary or clustered; sepals and petals each three, similar and petaloid, distinct or partly united; stamens six; anthers two-celled; pistil of three united carpels; ovary superior, three-celled; styles united; fruit a loculicidal capsule.

Flowers umbelloid 1. *Allium*
Flowers usually solitary, white 2. *Lloydia*
Flowers usually several, dark 3. *Fritillaria*

1. ALLIUM (Tourn.) L.

Scapose, bulbous plants with characteristic odor; leaves fleshy, usually narrowly linear but sometimes flat and broad, mostly basal; stems simple, erect; flowers in a terminal umbel, subtended by three membranous bracts; petals and sepals free or partly united at the base, one-nerved; stamens adnate to the base of the sepals and petals; styles filiform, usually deciduous; seeds black, one or two in each cell of the capsule. (Latin name of garlic.)

Leaves narrowly linear 1. *A. sibiricum*
Leaves broad 2. *A. victoralis*

1. *A. sibiricum* L. Wild Chives

Bulbs small, narrowly ovoid, clustered, membranous-coated; leaves very narrow, 10–35 cm. long; scapes 3–6 dm. tall, bearing a capitate umbel of rose-purple flowers; sepals and petals about 1 cm. long with dark midrib; capsule obtusely three-lobed, about half as long as the perianth. This species is closely related to the garden chives (*A. schoenoprasum* L.) and is sometimes rated as only a variety of it.

Widely distributed in Alaska and Yukon—Newf.—N. Y.—Wyo.—Ore. —Sib. (Fig. 321.)

2. *A. victoralis* L. ssp. *platyphyllum* Hult.

A vigorous form growing up to 75 cm. tall; leaves one or two, the blade thin and flat, elliptical or ovate, up to more than 2 dm. long and nearly 1 dm. wide, the sheath enclosing the base of the scape; flowers white; bulbs with reticulate cover.

An Asiatic form found on Attu Island. (Fig. 322.)

2. LLOYDIA Salisb.

Dwarf herb with tunicated bulb arising from a creeping rootstock; leaves very narrow and grass-like; flowers in our form usually reduced to one; sepals and petals nearly alike; stamens basi-fixed, dehiscent by marginal slits; ovary three-celled. (George Lloyd was an English naturalist.)

L. serotina (L.) Wats. Alp Lily

Bulbs small, covered with a grayish fibrous coat; stem slender, erect, 5–15 cm. tall; basal leaves 8–15 cm. long, about 1 mm. wide; stem leaves few, 1–4 cm. long, wider; flowers creamy white, about 1 cm. long, purple-veined and tinged with rose on the back; capsule ovoid, many-seeded, about 8 mm. long.

A circumpolar species widely distributed in Alaska. (Fig. 323.)

3. FRITILLARIA (Tourn.) L.

Simple leafy-stemmed plants from bulbs with thick scales; flowers campanulate, large, nodding; sepals and petals nearly equal, deciduous, each with a nectiferous pit at the base; anther attached at the base; style slender, three-cleft; seeds numerous, flat, winged. (Latin, chess-board, from the checkered marking of the perianth of some of the species.)

F. camtchatcensis (L.) Ker. Indian Rice. Black Lily

Bulb of several larger scales subtended by numerous rice-like bulblets; stems stout, simple, 3–6 dm. tall; leaves mostly in two or three whorls with a few scattered ones near the top, lanceolate, blunt, 5–9 cm. long; flowers one to six, dark-wine color, often almost black, tinged greenish-yellow on the outside, 18–30 mm. long; pod obtusely angled, 2–3 cm. long.

Along the coast, eastern Asia—Aleutian and Pribylof Islands—western Ore. (Fig. 324.)

13. CONVALLARIACEAE (Lily-of-the-valley Family)

Perennials with simple or branched rootstocks; leaves alternate or basal; flowers perfect, in axillary or terminal racemes or panicles or sometimes solitary; sepals and petals two, or more commonly three each, distinct or partly united; stamens four or six; gynoecium superior, of two or three united carpels; ovary two or three celled, styles united; fruit a berry. This group is often included in *Liliaceae*.

1A. Leaves all basal 1. *Clintonia*

2A. Stem more or less leafy.

1B. Perianth four-parted 2. *Maianthemum*

2B. Perianth six-parted.
1C. Flowers in a terminal raceme or panicle 3. *Smilacina*
2C. Flowers axillary.
1D. Perianth rotate 4. *Kruhsea*
2D. Perianth campanulate 5. *Streptopus*

1. CLINTONIA Raf.

Perennials with creeping rootstocks; leaves basal, broad, many-nerved; flowers borne on scapes; sepals and petals similar and petaloid; stamens six; anthers versatile, ovary two- or three-celled; style slender; berry ovoid or nearly globose. (Named for DeWitt Clinton, governor of New York.)

C. uniflora (Schultz) Kunth. Blue-bead

Leaves two to four, oblanceolate, more or less villous beneath, 1–2 dm. long, 3–6 cm. wide, acute at both ends; scape shorter than the leaves; flower white, campanulate, the sepals and petals about 2 cm. long; berry about 1 cm. long, five- to 10-seeded. The scape is occasionally two-flowered.

Woods, southeastern Alaska—Mont.—Calif. (Fig. 325.)

2. MAIANTHEMUM Weber.

Low perennials with slender rootstocks; leaves usually two or three, broad, many-nerved; flowers white, small, in a terminal raceme; sepals and petals each two, distinct and spreading; stamens four; anthers versatile; stigmas two; ovary two-celled; fruit a globose berry with one or two seeds. (Greek, May and flower, referring to the season of flowering.) *Unifolium* Haller.

M. dilitatum (Wood) Nels. & Macb. Deerberry
M. bifolium DC. var. *kamtschaticum* (Gmel.) Jeps.
U. dilitatum (Wood) Howell.
U. eschscholtzianum (Anders. & Bess.) Wight.

Stems 15–40 cm. tall, glabrous; leaves broadly cordate to sagittate, acuminate, 5–15 cm. long, 3–10 cm. wide, or those of the sterile stems up to 15 cm. wide; racemes many-flowered, the pedicels 2–4 mm. long, spreading, often fascicled; sepals and petals 2–3 mm. long, becoming reflexed, style stout; berry spotted, becoming red on drying.

Woods, eastern Asia—Aleutian Islands—southeastern Alaska—Ida.—Calif. (Fig. 326.)

3. SMILACINA Desf.

Perennials with slender, creeping rootstocks; stems leafy; flowers in terminal racemes or panicles; sepals and petals white or greenish-white, distinct or nearly so; stamens six; anthers introrse; ovary three-celled, style short, stigma three-lobed; berry globose. (Name a diminutive of Smilax.) *Vagnera* Adans.

Inflorescence a panicle 1. *S. racemosa*
Inflorescence a raceme 2. *S. stellata*

1. *S. racemosa* (L.) Desf. Wild or False Spikenard
V. racemosa (L.) Morong.

Rootstocks fleshy; stem somewhat angled, 3–10 dm. tall; leaves oblong-lanceolate, sessile or short-petioled, 7–20 cm. long, pubescent below with short, stiff hairs, the margins minutely ciliate; panicle densely many-flowered, 4–10 cm. long; sepals and petals oblong, about 2 mm. long; berries speckled with purple, 4–6 mm. in diameter.

Hyder—N. S.—Geo.—Ariz.—B. C. (Fig. 327.)

2. *S. stellata* (L.) Desf. Star-flowered Solomon's Seal
V. stellata (L.) Morong.

Stem more or less flexuous above, 3–5 dm. long; leaves sessile, pubescent beneath, lanceolate, many-nerved, 5–15 cm. long, 2–4 cm. wide; racemes 3–7 cm. long, several-flowered; sepals and petals about 6 mm. long; berries 7–10 mm. in diameter.

Cook Inlet district—Mont.—Utah—Calif. (Fig. 328.)

4. KRUHSEA Regel.

A low, glabrous perennial with slender rootstocks; flowers solitary, extra-axillary, the perianth rotate, deeply wine-colored with greenish reflexed tips; stamens six, the filaments very short; ovary three-celled, becoming a bright red, globose berry. (Named for Dr. Kruhse of Siberia.)

K. streptopoides (Ledeb.) Kearney. Kruhsea
Streptopus streptopoides (Ledeb.) Frye & Rigg.

Stem simple, 3–15 cm. tall; leaves four to eight, sessile, ovate-lanceolate, acute, 25–50 mm. long; flowers one to five, on recurved pedicels scarcely 1 cm. long; sepals and petals 2–2.5 mm. long.

Woods, eastern Asia, central and southeastern Alaska—Ida.—Wash. (Fig. 329.)

5. STREPTOPUS Michx.

Leafy perennials; leaves thin, many-nerved, sessile or clasping; flowers usually solitary, extra-axillary; peduncles slender, twisted or bent above the middle; sepals and petals nearly alike, recurved, deciduous, petals keeled; stamens six; anthers sagittate, extrorse; ovary three-celled, stigma three-lobed; berry globose or oval, many-seeded. (Greek, twisted-stalk, referring to the peduncles.)

Leaves clasping, flowers greenish 1. *S. amplexifolius*
Leaves sessile, flowers pinkish 2. *S. roseus*

1. *S. amplexifolius* (L.) DC. Cucumber-root. Clasping Twisted-stalk

Rootstock short, stout, horizontal, with thick fibrous roots; stems usually branched, 3–10 dm. tall; leaves ovate-lanceolate, acuminate, strongly clasping, glaucous beneath, 5–12 cm. long; flowers campanulate, sepals and petals 8–12 mm. long, attenuate; berry ellipsoid, 10–15 mm. long, yellowish-white to light red.

Most of our area south of the Arctic Circle; circumboreal. (Fig. 330.)

2. *S. roseus* Michx. ssp. *curvipes* (Vail) Hult.
Simple-stemmed Twisted-Stalk

Rootstocks slender with fibrous roots; stem usually simple, 1–4 dm. tall; leaves sessile, oblong-lanceolate, acuminate, 4–10 cm. long, the margins more or less short-ciliate; sepals and petals pinkish, 5–7 mm. long, glandular-pubescent on the inner surface; peduncles glandular, not geniculate; fruit red, globose, 7–9 mm. in diameter.

Woods, southeastern Alaska and northwestern B. C.—Wash. Other forms in eastern U. S. and Canada. (Fig. 331.)

14. IRIDACEAE (Iris Family)

Perennials arising from bulbs or commonly from rootstocks; leaves equitant, often grass-like; flowers bracted, perfect, regular or nearly so, sepals and petals three each, often dissimilar but both colored; stamens three, opposite the sepals; gynoecium of three-united carpels with three-celled, inferior ovary and three distinct styles; fruit a loculicidally dehiscent capsule.

Flowers more than 5 cm. wide 1. *Iris*
Flowers less than 25 mm. wide 2. *Sisyrinchium*

1. IRIS (Tourn.) L.

Perennials with sword-shaped or linear leaves; flowers large and showy in few-flowered terminal clusters; perianth in ours blue, the sepals spreading or recurved, the petals smaller and erect or ascending, the tube prolonged beyond the ovary; the three styles are petal-like and arch over the stamens; capsule elongated, three or six angled with numerous seeds. (Greek, rainbow, referring to the colored flowers.)

I. setosa Pall. Wild Iris, Flag
I. arctica Eastw.

Stems densely tufted, 35–70 cm. tall; basal leaves linear-lanceolate, 2–5 dm. long, 5–12 mm. wide; stem leaves two or three, the stem usually with one branch; perianth blue varying to lavender and purple, the sepals 5–6 cm. long, copiously veined, petals cuspidate; style branches large, crested; capsule oblong, 3–4 cm. long. Var. *platyrincha* Hult. has the petals broad, flat, dilated below and constricted in the middle. Ssp. *interior* (E. Anderson) Hult. is the form found in the interior. It differs from the coast form in having narrower and less arched leaves, and in more scarious and somewhat violet-colored bracts shorter than the peduncles.

Siberia—Alaska—Labr.—Newf.—Maine. (Fig. 332.)

2. SISYRINCHIUM L.

Grass-like, tufted perennials, usually with winged stems; flowers usually blue, borne in a few-flowered terminal umbel subtended by a pair of erect green bracts; perianth-tube short or none; sepals and petals similar, spreading, aristulate; capsule globose or ovoid, three-valved, dehiscent. (Name used by Theophrastus for a plant allied to Iris.)

S. littorale Greene. Blue-eyed Grass

Stems 2–4 dm. tall, prominently winged; leaves 8–25 cm. long, less than 5 mm. wide; umbels three to six flowered, sepals and petals about 11 mm. long; capsules subglobose, 6–8 mm. long, thick-walled.

Wet soil. Pacific Coast Districts of Alaska and B. C. (Fig. 333.)

15. ORCHIDACEAE (Orchid Family)

Perennials with corms, bulbs, or tuberous roots; leaves simple, entire, sheathing, often reduced to scales; flowers irregular, perfect, bracted, solitary or in racemes or spike; sepals three, similar or nearly so, the lower two sometimes united; petals three, the lateral ones alike, the third (lip) differing, usually very markedly so, often spurred, usually inferior by the twisting of the ovary; functional stamens two in Cypripedium, one in the other genera, adnate to the pistil and forming a column, two-celled and containing two or more waxy or powdery pollinia which are usually stalked and attached at the base to a viscid gland; style often terminating in a beak (rostellum) at the base of the anther or between its sacs; ovary inferior, usually long and twisted, three-angled, one-celled with three parietal placentae and numerous ovules; seeds vary numerous and minute. A very large and interesting family of plants. Many tropical species are epiphytes, attached to the limbs of trees but not parasitic, deriving nutriment from the air and moisture alone, being assisted in this by the symbiotic fungi living in the roots, corms or bulbs. The flowers are so constructed that they are dependent on insects for pollination, the head of the insect when reaching for the nectar comes in contact with the bases of the pollinia which adhere to its head, and when visiting the next flower the pollinia adhere to the sticky stigma and the process is repeated.

1A. Fertile stamens two, lip saccate 1. *Cypripedium*
2A. Fertile stamen one.
1B. Pollinia caudate at the base, attached to a viscid gland.
1C. Gland enclosed in a pouch-like fold, lip three-lobed 2. *Orchis*
2C. Gland surrounded by a thin membrane, lip toothed at the apex 3. *Coeloglossum*
3C. Gland naked, lip entire.
1D. Sepals one-nerved, plants with corms..... 7. *Piperia*
2D. Sepals three to five nerved, plants with rootstocks.
1E. Stem leafy 6. *Limnorchis*
2E. Stems scapiform, leaves one or two, basal.
1F. Leaves two, ovary straight 4. *Lysias*
2F. Leaf one, ovary curved 5. *Lysiella*
2B. Pollinia not caudate at the base.
1C. Pollinia granulose or powdery.
1D. Leaves basal, usually white-reticulate.....10. *Peramium*
2D. Leaves green, borne on stem.
1E. Leaves alternate, spike twisted 8. *Spiranthes*
2E. Leaves two, opposite 9. *Listera*

2C. Pollinia smooth or waxy.
1D. Plants with coralloid roots 13. *Corallorrhiza*
2D. Plants with corms.
1E. Lip flat, flowers in a raceme 11. *Malaxis*
2E. Lip saccate, flowers usually one 12. *Calypso*

1. CYPRIPEDIUM L.

Glandular-pubescent herbs with coarse, fibrous roots; leaves large and broad, somewhat plaited, many-nerved; flowers in ours solitary, showy; sepals spreading or two of them united; lip a large inflated sac; column incurved, concealed by a recurving petaloid sterile stamen; capsule ribbed. (Greek, Venus and shoe.)

1A. Leaves two, lip pink 1. *C. guttatum*
2A. Leaves several.
1B. Flowers large, sepals longer than lip 2. *C. montanum*
2B. Flowers small, sepals shorter than lip 3. *C. passerinum*

1. *C. guttatum* Sw. Pink Ladies' Slipper

Rootstock horizontal; the two-leaved stems 12–24 cm. tall; leaves lanceolate, 6–11 cm. long; lip 18–25 mm. long, pink spotted purplish and veined, longer than the sepals; capsule glandular-pubescent, strongly ribbed, reflexed.

Aleutian Islands—Yukon Valley—Great Bear Lake—Great Slave Lake and Eurasia. (Fig. 334.)

2. *C. montanum* Dougl. Mountain Ladies' Slipper

Stems 3–7 dm. tall; leaves ovate to broadly lanceolate, 8–16 cm. long, 4-8 cm. wide, abruptly acuminate; sepals lanceolate, 4–6 cm. long, the petals similar but narrower; lip oblong, 2–3 cm. long, white veined with purple; capsule erect or nearly so, 2–3 cm. long.

Glacier Bay, the Stickine River, and the Lewes River in Yukon—B. C.—Sask—Wyo.—Calif.

3. *C. passerinum* Richards. Northern Ladies' Slipper

Stems 1–3 dm. tall, often retrosely villous; leaves oval or lanceolate, 6–15 cm. long; lip about 15 mm. long, whitish with purplish spots inside; sepals 10–15 mm. long, the lower slightly two-cleft; capsule upright.

Woods, Seward Peninsula—Yukon valley—Alta.—Ont. (Fig. 335.)

2. ORCHIS (Tourn.) L.

Sepals distinct, spreading; petals narrower than the sepals; lip spurred; anther of two pollen masses, slightly diverging, prolonged into a slender stalk attached to a small gland which is enclosed in a pouch. (Ancient name.)

Stem leafy 1. *O. aristata*
Stem scapose with one basal leaf 2. *O. rotundifolia*

1. *O. aristata* Fisch. Rose-purple Orchis

Stems 12–40 cm. tall, from thick, fusiform, forked tubers; leaves

obovate to lanceolate, up to 12 cm. long and 25 mm. wide; lower bracts leaf-like; flowers several to many, rose-purple, in a spike-like raceme 4–10 cm. long; lip very broad, the middle lobe acute; sepals and petals acuminate to aristate; spur large.

Eastern Asia—Aleutian Islands—Nunivak Island—Pr. William Sound. (Fig. 336.)

2. *O. rotundifolia* Pursh. Round-leaved Orchis

Stem slender, 8–16 cm. tall, arising from a rootstock with fibrous roots; leaf solitary, near the base, oval to orbicular, 2–6 cm. long; spike two to six flowered; flowers 12–15 mm. long, subtended by bracts, sepals elliptic, pink, 6–7 mm. long, petals narrower; lip white, purple-spotted, the large middle lobe notched at the apex; spur slender, curved, shorter than the lip.

Central Alaska—Greenl.—Maine—N. Y.—Minn.—Alta.—B. C. (Fig. 337.)

3. COELOGLOSSUM Hartm.

Tubers two to three cleft; leaves alternate; flowers in terminal spikes; sepals distinct, converging, forming a hood; lateral petals narrow, erect; lip obtuse, two or three toothed at the apex, prolonged below into a sac-like spur. Included by some authors in Habenaria. (Latin, Heaven-tongue.)

C. viride (L.) Hartm. Long-bracted Orchid

H. viridis var. *interjecta* Fern.

Leaves ovate, obovate or lanceolate; bracts linear-lanceolate, the lower ones usually about twice as long as the flowers; sepals ovate-lanceolate, petals narrow; lip 5–8 mm. long, oblong or somewhat cuneate. The typical form growing only 6–15 cm. tall with two or three stem-leaves is found on Seward Peninsula and Central Alaska. Much more frequent is the ssp. *bracteatum* (Muhl.) Hult. (*C. bracteatum* (Muhl.) Parl. (*H. bracteata* (Muhl.) R. Br.) found in the Pacific Coast districts. It grows 1–4 dm. tall and has three to five stem leaves.

The species is circumboreal. (Fig. 338.)

4. LYSIAS Salisb.

Plants with fleshy rootstocks or tubers; leaves two, near the base, broad; flowers in a terminal spike, greenish or white; sepals distinct, large, spreading, the upper one broadly cordate, the lateral ones obliquely ovate, lateral petals small and narrow; lip entire, narrow, prolonged at the base into a slender spur. Included in Habenaria by some authors, in Platanthera by others. (Lysias was an Attic orator.)

L. orbiculata (Pursh.) Rydb. Large Round-leaved Orchid

H. orbiculata (Pursh.) Torr.

P. orbiculata (Pursh.) Lindl.

Scapes 3–4 dm. tall with one or two lanceolate bracts above the basal

leaves; leaves 7–12 cm. long, 4–8 cm. wide; sepals 7–8 mm. long; lip linear, 8–10 mm. long; spur 15–20 mm. long; anther-sacs large and prominent.

Rare, southeastern Alaska—Newf.—S. Car.—Ill.—Wash. (Fig. 339.)

5. LYSIELLA Rydb.

Small plants with scapiform stems; leaf solitary, basal; flowers greenish-yellow; upper sepal round–ovate, erect, surrounding the column, lateral sepals reflexed-spreading; lip entire, linear-lanceolate, deflexed; spur slightly curved, shorter than the ovary; capsule ovoid. (Diminutive of *Lysias*.) Often included in Habenaria or Platanthera.

L. obtusata (Pursh.) Rydb. Small Northern Bog Orchid
H. obtusata (Pursh.) Rich.
P. obtusata (Pursh.) Rich.

Stem slender, glabrous, 8–25 cm. tall; leaf 5–10 cm. long; flowers four to twelve, about 1 cm. long; spur slender, about as long as the lip and nearly as long as the ovary.

Widely distributed in Alaska—Labr.—Newf.—N. Y.—Colo.—B. C. (Fig. 340.)

6. LIMNORCHIS Rydb.

Leafy-stemmed plants with fusiform, root-like tubers; flowers small, greenish or white, borne in a terminal spike; upper sepal ovate to sub-orbicular, erect, three to seven nerved, lateral sepals linear to ovate-lanceolate, usually three nerved; lateral petals erect, usually lanceolate and three nerved; lip entire, reflexed, from linear to rhombic lanceolate, or orbicular in one species; column short and thick; anther-sacs parallel. This group is included in Habenaria by some authors and in Platanthera by others. Some forms are hard to place and there has been much confusion regarding the species. It seems evident that several of the species hybridize readily resulting in much natural variation. No two writers seem to agree on the limits of the species and the same name has been used in literature for different forms. (Greek, marsh and orchid.)

1A. Floral parts about 1 mm. long 1. *L. chorisiana*
2A. Floral parts much longer.
 1B. Spur at least twice as long as the lip 2. *L. behringiana*
 2B. Spur one-half to one and one-half as long as the lip.
 1C. Lip linear.
 1D. Spike rather dense 3. *L. convallariaefolia*
 2D. Spike lax 4. *L. stricta*
 2C. Lip distinctly broader at the base.
 1D. Lip obtusely triangular 5. *L. hyperborea*
 2D. Lip linear with dilated base 6. *L. dilitata*

1. *L. chorisiana* (Cham.) new comb. Choriso Bog-orchid
H. chorisiana (Cham.)
P. chorisiana (Cham.) Rchb.

Stems 10–15 cm. tall; tuber elongated-fusiform, leaves two at or near

the base, 25–40 mm. long, 8–20 mm. wide, with usually a bract-like leaf on the stem above; bracts lanceolate, longer than the flowers; flowers very small, the parts scarcely more than 1 mm. long; capsule about 5 mm. long.

Not common, eastern Asia, Aleutian Islands, and on Douglas Island. (Fig. 341.)

2. *L. behringiana* Rydb. Bering Bog-orchid

P. behringiana (Rydb.) Tatew. & Kobay.

Stems 10–15 cm. tall; tubers elongate fusiform; main leaf about 5 cm. long, 15–20 mm. wide, and usually two lanceolate smaller ones; spike 3–4 cm. long; bracts linear-lanceolate, the lowest about twice as long as the flowers; flowers purplish; lip about 5 mm. long; spur fully 1 cm. long. Hultén considers this as only a dwarf form of *Platanthera tipuloides* (L.f.) Lindl. of Asia.

In America known only from Attu Island.

3. *L. convallariaefolia* (Fisch.) Rydb.

Stems 2–6 dm. tall; tuber fusiform, moderately elongated; leaves 4–6 cm. long, 10–22 mm. wide, the lower obtuse, the upper acute; spikes 5–12 cm. long; flowers greenish or sometimes whitish; lip linear; spur about equaling the lip, linear or clavate. This species seems to hybridize with *L. dilitata* and *L. hyperborea*. Var. *dilitatoides* is probably such a hybrid. It is a rather robust plant with whitish flowers and the lip dilated at the base.

Eastern Asia—Aleutian Islands—Cook Inlet. (Fig. 342.)

4. *L. stricta* (Lindl.) Rydb. Slender Bog-orchid

H. saccata Greene.

P. stricta Lindl.

Stems 2–10 dm. tall; lower leaves lanceolate, obtuse, upper leaves smaller, acute; spikes 1–3 dm. long, lax; bracts linear-lanceolate, the lower much longer than the flowers; flowers greenish, upper sepal erect, ovate, 4–5 mm. long; spur saccate, shorter than the lip.

Common in the Pacific coastal districts of Alaska—Alta.—Colo.—Calif. (Fig. 343.)

5. *L. hyperborea* (L.) Rydb. Northern Bog-orchid

H. hyperborea (L.) R. Br.

P. hyperborea (L.) Lindl.

Stem 15–50 cm. tall; lower leaves oblanceolate, obtuse, upper leaves lanceolate and acute; spike rather dense, 4–10 cm. long; flowers light green, upper sepal ovate, 3–4 mm. long; lip triangular-ovate, obtuse, 3–5 mm. long; spur clavate, curved, about equaling the lip.

Central Alaska—Labr.—Iceland—Pa.—Colo.—S. C. (Fig. 344.)

6. *L. dilitata* (Pursh.) Rydb. White Bog-orchid

H. dilitata (Pursh.) Hook.

P. dilitata (Pursh.) Lindl.

Stems 2–8 dm. tall; tubers elongated-fusiform; lower leaves lanceo-

late, often obtuse, the upper narrower and acute, 8–20 mm. wide; flowers white; lip linear with distinctly dilated base, the margins papillose; spur linear, about the same length as the lip. The above description applies to the more typical forms. Var. *angustifolia Hook.* (*L. leptoceratatis* Rydb.) Leaves not over 8 mm. wide; tuber slender and elongated; inflorescence rather lax; spur one to two times as long as the lip. Var. *chlorantha* Hult. Base of lip dilated, the margins sparsely papillose; flowers greenish; leaves wide. Var. *leucostachys* (Lindl.) Ames. More robust; inflorescence dense; flowers white, very fragrant; tuber thicker and less elongated; spur fully one-half longer than the lip. This is the form known as Wild Hyacinth.

Pacific Coast districts of Alaska; across the continent in some of its forms. (Fig. 345.)

7. PIPERIA Rydb.

Stems arising from spherical or broadly ellipsoid tubers; leaves few, near the base, usually withering at or shortly after anthesis; flowers small, spicate; upper sepal erect, the lateral spreading; lateral petals free, oblique; lip linear-lanceolate to ovate, concave, united with the base of the lower sepals; anther-sacs parallel. Often included in Habenaria or Platanthera. (Charles V. Piper was a botanist of Washington State and the U. S. Dept. of Agriculture.)

P. unalaschensis (Spreng.) Rydb. Alaska Piperia
H. unalaschensis (Spreng.) Wats.
Platanthera unalaschensis (Spreng.) F. Kurtz.

Stem slender, 3–5 dm. tall; basal leaves oblanceolate, the largest 10–15 cm. long, stem leaves bract-like; spike lax, 1–3 dm. long; flowers greenish, 8–14 mm. long; sepals and petals 2–4 mm. long, upper sepal ovate; lip oblong, obtuse; spur narrow, rather longer than the lip.

Unalaska, southeastern Alaska—Que.—Colo.—Calif. (Fig. 346.)

8. SPIRANTHES L. C. Richards

Herbs with tuberous-thickened or fleshy-fibrous roots; leaves alternate or mostly basal; flowers in twisted spikes, white or cream-colored, small, spurless, sepals and petals in ours more or less united or connivant into a hood; lip concave, small, dilated at the reflexed apex; column oblique, arched; pollinia two; stigma with a beak. (Name from the spiral arrangement of the flowers.) (*Ibidium* Salisb.) (*Gyrosrachys* Pers.)

S. romanzoffiana Cham. & Schleicht. Hooded Ladies' Tresses
I. romanzoffianum (C. & S.) House.

Stems 7–30 cm. tall; lower leaves linear to lanceolate, 6–15 cm. long; spikes dense, 4–10 cm. long, the flowers in three spiral rows; bracts often longer than the flowers; lip oblong, broad at the base, contracted above the dilated, cusped apex.

Wet soil, Unalaska—Labr.—Newf.—N. Y.—Colo.—Calif., and in Ireland. (Fig. 347.)

9. LISTERA R. Br.

Slender woodland plants; rootstocks with fleshy-fibrous roots; leaves two, opposite, near the middle of the stem; flowers small, greenish or purplish, spurless, in terminal racemes; sepals and lateral petals similar, spreading or reflexed; lip longer than the sepals; pollinia two, united to a minute gland; capsule ovoid or obovoid. (Martin Lister was an English naturalist.) (*Ophrys* (Tourn.) L.)

1A. Lip narrow, deeply cleft 4. *L. cordata*
2A. Lip broad, slightly cleft or notched at apex.
1B. Lip with auricles 1. *L. borealis*
2B. Lip without auricles but with small teeth at base.
1C. Ovary glandular 2. *L. convallariodes*
2C. Ovary glabrous 3. *L. caurina*

1. *L. borealis* Morong. Northern Twayblade
O. borealis (Morong) Rydb.

Stems 6–15 cm. tall; leaves 10–35 mm. long, rather firm, elliptic ovate, obtuse, borne above the middle of the stem; flowers two to six, 10–12 mm. long; lip 7–8 mm. long, oblong-cuneate, the lobes at the apex obtuse and without mucro; column 3–4 mm. long.

Central Alaska—Mack.—Colo. (Fig. 348.)

2. *L. convallarioides* (Sw.) Torr. Broad-leaved Twayblade
O. convallarioides (Sw.) Rydb.

Stems 10–25 cm. tall, glandular-pubescent above the leaves; leaves broadly oval or suborbicular, obtuse or very short-cuspidate, 3–6 cm. long; flowers greenish-yellow on short, slender, bracted pedicels; sepals linear-lanceolate; lip broadly cuneate, 7–10 mm. long with two obtuse lobes at the apex and a mucro between; ovary glandular and pubescent.

Woods, Aleutian Islands and B. C.—Newf.—Mass.—N. Mex. (Fig. 349.)

3. *L. caurina* Piper Western Twayblade
O. caurina (Piper) Rydb.

Stems 1–3 dm. tall; leaves short-elliptic to ovate, 3–7 cm. long; lip 4–7 mm. long, cuneate, retuse with a blunt mucro in the sinus; ovary glabrous. Resembles *L. convallarioides* in appearance but is a more slender plant, has narrower leaves, longer pedicels and smaller flowers. The pedicels are two to four times as long as the bracts. This is the common species in southeastern Alaska.

Alaska—Mont.—Ore. (Fig. 350.)

4. *L. cordata* (L.) R. Br. Heart-leaved Twayblade
O. cordata L.

Stem slender and delicate, 1–2 dm. tall, glabrous except just above the leaves; leaves cordate-reniform, mucronate, 15–35 mm. long and about as wide; racemes four to twenty flowered, bracts minute, pedicels about 2 mm. long; flowers greenish or purplish, sepals and petals about 2 mm.

long; lip narrow, 4–5 mm. long, the segments setaceous; capsule ovoid, about 4 mm. long. Our western form has broader leaves than the type and if regarded as separate is the var. *nephrophylla* (Rydb.) Hult. (*L. nephrophylla* Rydb.) (*O. nephrophylla* Rydb.).

Woods, common, Pacific coastal districts of Alaska; circumboreal. (Fig. 351.)

10. PERAMIUM Salisb.

Herbs with creeping rootstocks and fleshy-fibrous roots; leaves basal, variegated, evergreen, strongly reticulated; flowers white or cream-colored, in one-sided racemes on scape-like, bracted stems; lateral sepals distinct, the upper united with the lateral petals; lip concave or saccate, roundish ovate with reflexed tip; anther with two pollinia attached to a small disc; inflorescence glandular. (Greek, referring to the pouch-like lip.) (*Goodyera* R. Br.)

Lip concave, the margins involute 1. *P. decipiens*
Lip saccate, the margins revolute 2. *P. repens*

1. *P. decipiens* (Hook.) Piper. Menzies Rattlesnake Plantain
P. menziesii (Lindl.) Morong.
G. decipiens (Hook.) Hubbard.

Scape rather stout, 2–4 dm. tall, glandular-pubescent; leaves ovate-lanceolate, 4–8 cm. long, acute at both ends, usually whitish along the veins; perianth 7–9 mm. long; anther ovate, long-pointed.

Woods, southeastern Alaska—Que.—N. Hamp.—Minn.—Ariz.—Calif. (Fig. 352.)

2. *P. repens* (L.) Salisb. var. *ophioides* Fern. Lesser Rattlesnake Plantain
G. repens (L.) R. Br.

Appearing like a miniature of *P. decipiens*. Scapes 1–2 dm. tall; leaves ovate with whitish blotches, 10–25 mm. long, tapering into a sheathing petiole; perianth greenish-white, scarcely 4 mm. long; anther blunt; lip with a narrow recurved or spreading apex; column short.

Woods, central Alaska—Labr.—Newf.—N. Car.—N. Mex.—the whole species circumboreal. (Fig. 353.)

11. MALAXIS Soland.

Perennials with corms; leaves one to four, on lower part of stem; flowers small, in terminal spike-like racemes, whitish or greenish; sepals spreading, distinct; lip embracing the column; anther two-celled with 4 pollinia, without tail or glands. (Greek, in allusion to the soft tissue.) (*Microstylis* Nutt.) (*Acroanthes* Raf.)

Plants small, leaves two or four 1. *M. paludosa*
Plants larger, leaves one or two 2. *M. monophylla*

1. *M. paludosa* (L.) Sw. Little Adder's Mouth

Stems 3–12 cm. tall; leaves ovate, 6–20 mm. long; flowers about 6 mm.

long; lip about 3.5 mm. long, narrow, slightly tapering toward the rounded apex; pedicels ascending; capsule 3–4 mm. long.

Muskeags, southeastern Alaska and scattered circumboreal stations. (Fig. 354.)

2. *M. monophylla* (L.) Sw. White Adder's Tongue
Microstylis monophyllos (L.) Lindl.

Stems glabrous, striate, 10–25 cm. tall; main leaf one, the blade 3–8 cm. long, 1–3 cm. wide, with usually a second leaf that may vary from scale-like to nearly as large as the main leaf; sepals and lip about 2 mm. long; lip 1.5 mm. wide; capsule 4–5 mm. long, 3–4 mm. wide.

Unalaska eastward along the coast; circumboreal. (Fig. 355.)

12. CALYPSO Salisb.

Low, scapose, one-flowered plants; corm superficial; leaf solitary, basal, flower terminal, showy; sepals and lateral petals similar, spreading or ascending, oblong-lanceolate, pinkish, lip large, saccate, spotted brown-purple, hairy within, with two short spurs near the apex; column winged, petal-like, with the anther just below the summit; pollinia two in each sac. (Greek god, Calypso, whose name signifies concealment.) *Cytherea* Salisb.

C. bulbosa (L.) Rchb. Calypso
Cytherea bulbosa (L.) House.

Scape 5–15 cm. tall; leaf ovate, 25–50 mm. long, 15–30 mm. wide, with a subcordate base; flowers variegated purple, pink, yellow; petals, sepals and lip 15–20 mm. long.

Mossy woods, rather rare except on some small islands, central Alaska south and east, nearly circumboreal. (Fig. 356.)

13. CORALLORRHIZA R. Br.

Brownish, purplish, or yellowish saprophytic plants; rootstocks coral-like masses from which the bracted, scape-like stems arise; flowers in terminal racemes; sepals nearly equal, the lateral ones united with the foot of the column and often forming a short spur, partly or wholly adnate to the top of the ovary; lip one to three ridged; anther terminal, lid-like, with four waxy pollinia. (Greek, meaning coral and root.)

Sepals and petals one-nerved 1. *C. trifida*
Sepals and petals three-nerved 2. *C. mertensiana*

1. *C. trifida* Chatelin. Early Coral-root
C. innata R. Br.
C. corallorrhiza (L.) Karst.

Stems slender, glabrous, 1–3 dm. tall with two or three sheathing bracts; flowers greenish-yellow or greenish-brown; sepals and petals about 5 mm. long; lip shorter than the petals, whitish, two-lobed; spur a small protuberance adnate to the top of the ovary; capsule reflexed, 8–12 mm. long.

Circumboreal; in our area from Kotzebue Sound south and east. (Fig. 357.)

2. *C. mertensiana* Bong. Mertens Coral-root

Stems purple or brownish-purple, with two or three sheathing bracts, 2–5 dm. tall; flowers ten to twenty-five, sepals nearly 1 cm. long, the petals slightly shorter; lip with two sharp teeth at about the middle, crenulate, somewhat spotted, about same length as the sepals, narrowed at the base; column about 7 mm. long, spur free from the ovary at the apex; capsule 15–18 mm. long, narrowed at the base, reflexed.

Coniferous forest, southeastern Alaska—Mont.—Wyo.—Calif. (Fig. 358.)

PLATE XI

Fig.

192. *Rynchospora alba* (L.) Vahl. Inflorescence, scale and achene.
193. *Eleocharis acicularis* (L.) R. & S. Spike, achene and style.
194. *Eleocharis kamtschatica* (C. A. Mey.) Kom. Spike and achene.
195. *Eleocharis uniglumis* (Link.) Schult. Spike and achene.
196. *Eleocharis palustris* (L.) R. & S. Spike and achene.
197. *Eriophorum alpinum L.* Spike and achene.
198. *Eriophorum scheuchzeri* Hoppe. Achene.
199. *Eriophorum chamissonis* C. A. Mey. Achene.
200. *Eriophorum vaginatum* L. Achene and upper sheath.
201. *Eriophorum brachyantherum* Trautv. Achene and upper sheath.
202. *Eriophorum gracile* Koch. Achene.
203. *Eriophorum angustifolium* Roth. Achene.
204. *Scirpus caespitosus* L. var. *callosus* Bigel. Spike and achene.
205. *Scirpus americanus* Pers. Inflorescence, cross section of stem, scale and achene.
206. *Scirpus pacificus* Britt. Inflorescence, scale and achene.
207. *Scirpus rufus* (Huds.) Schrad. Inflorescence, spike and achene.
208. *Scirpus validus* Vahl. Cross section of stem, portion of inflorescence, achene and style.
209. *Scirpus microcarpus* Presl. Achene, portion of inflorescence and scale.
210. *Kobresia myosuroides* (Vill.) Fiori & Paol. Inflorescence and achene.
211. *Carex nardina* Fr. Achene and scale.
212. *Carex jacobi-peteri* Hult. Scale, achene and perigynium.
213. *Carex capitata* L. Achene, scale and perigynium.

PLATE XI

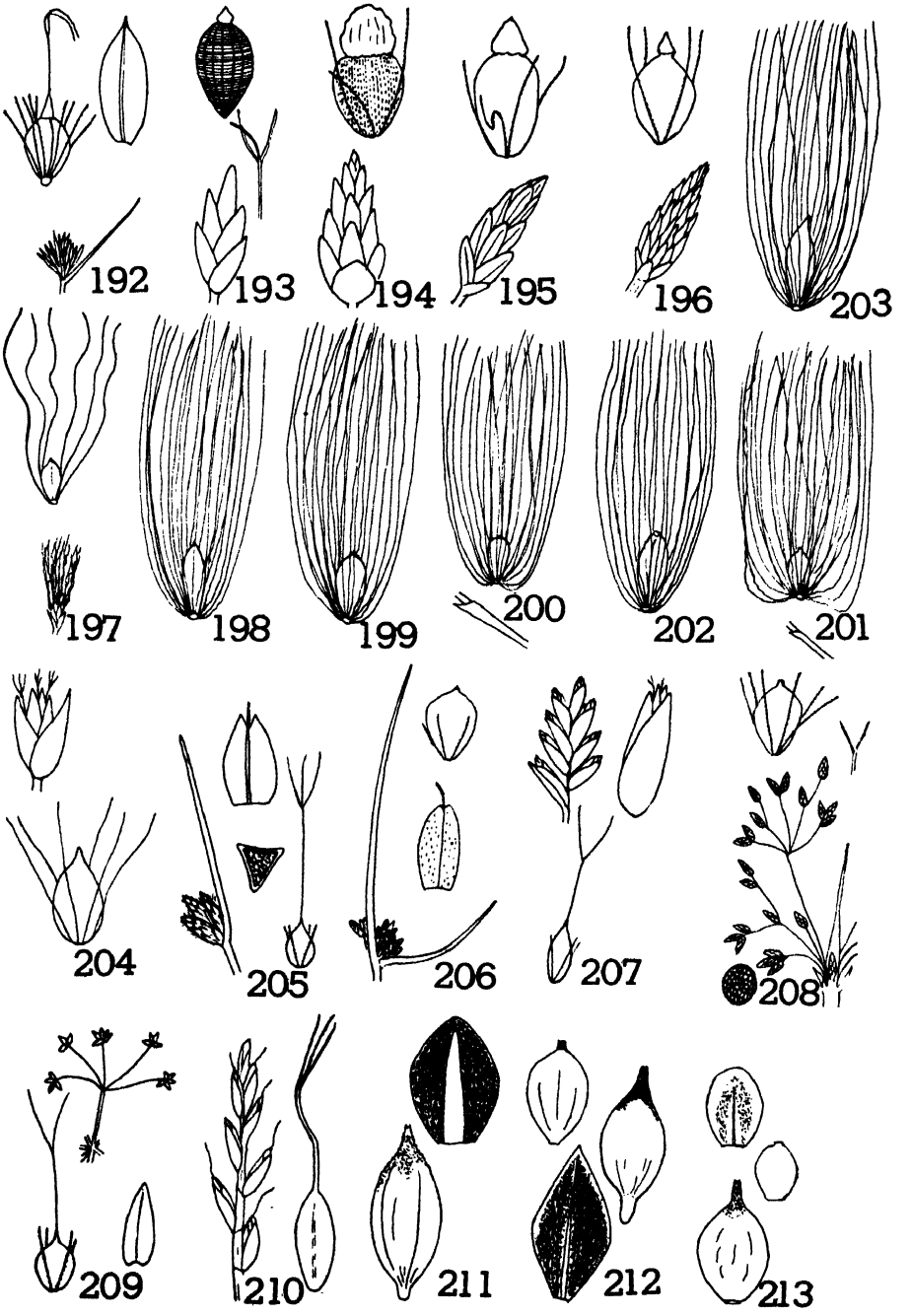

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PLATE XII.

All these illustrations except fig. 216 and 218 show scale, perigynium and achene.

Fig.

214. *Carex gynocrates* Wormskj.
215. *Carex scirpoidea* Michx.
216. *Carex anthoxanthea* Presl.
217. *Carex circinata* C. A. Mey.
218. *Carex leptalea* Wahl. Top of spike, scale and perigynium.
219. *Carex obtusata* Lilj.
220. *Carex pyrenaica* Wahl.
221. *Carex nigricans* C. A. Mey.
222. *Carex pauciflora* Lightf.
223. *Carex microglochin* Wahl.
224. *Carex maritima* Gunner.
225. *Carex chordorrhiza* Ehrh.
226. *Carex stipata* Muhl.
227. *Carex diandra* Schk.
228. *Carex athrostachya* Olney.
229. *Carex macloviana* d'Urv. ssp. *pachystachya* (Cham.) Hult.
230. *Carex praticola* Rydb.
231. *Carex crawfordii* Fern.
232. *Carex aenea* Fern.
233. *Carex lachenalii* Schk.
234. *Carex pribylovensis* Macoun.
235. *Carex glareosa* Wahl.
236. *Carex mackenziei* Kretch.
237. *Carex canescens* L.
238. *Carex brunnescens* (Pers.) Poir.
239. *Carex disperma* Dewey.
240. *Carex tenuiflora* Wahl.
241. *Carex loliacea* L.
242. *Carex stellulata* Good.
243. *Carex phyllomanica* W. Boott.

PLATE XII.

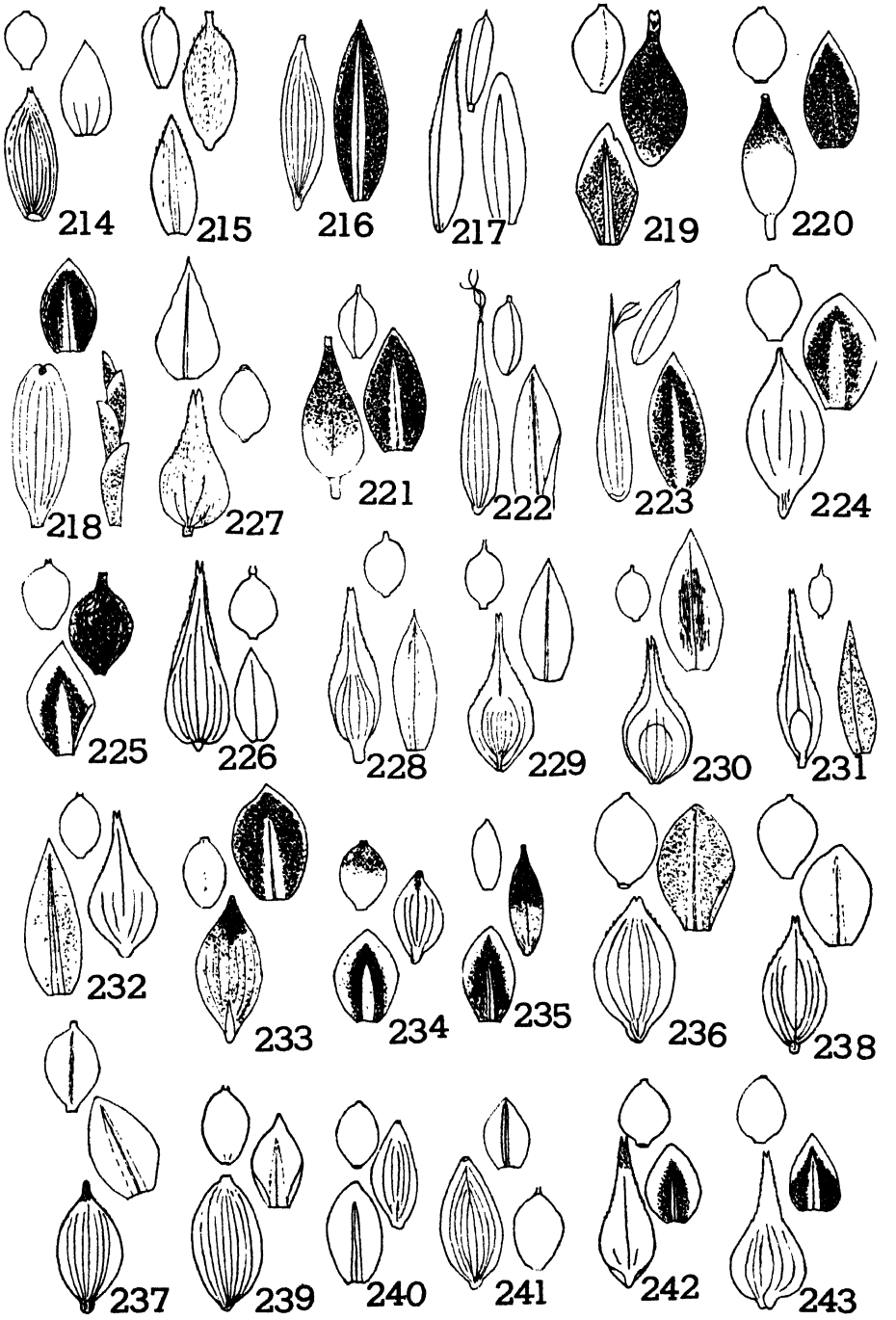

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PLATE XIII

All illustrations of Carex show scale, perigynium and usually the achene.

Fig.

244. *Carex laeviculmis* Meinsh.
245. *Carex bicolor* All.
246. *Carex aurea* Nutt.
247. *Carex garberi* Fern. ssp. *bifaria* Fern.
248. *Carex bigelowii* Torr.
249. *Carex lugens* Holm.
250. *Carex kelloggii* W. Boott.
251. *Carex hindsii* C. B. Clarke.
252. *Carex aquatilis* Wahl.
253. *Carex sitchensis* Prescott.
254. *Carex subspathacea* Wormskj.
255. *Carex ramenskii* Kom.
256. *Carex lyngbyei* Hornem. ssp. *cryptocarpa* (C. A. Mey.) Hult.
257. *Carex norvegica* Retz. ssp. *inferalpina* (Wahl.) Hult.
258. *Carex buxbaumii* Wahl.
259. *Carex stylosa* C. A. Mey.
260. *Carex gmelini* Hook. & Arn.
261. *Carex atrata* L.
262. *Carex mertensii* Prescott.
263. *Carex macrochaeta* C. A. Mey.
264. *Carex montanensis* Bailey.
265. *Carex spectabilis* Dewey.
266. *Carex nesophila* Holm.
267. *Carex podocarpa* R. Br.
268. *Carex deflexa* Hornem.
269. *Carex rossii* Boott.
270. *Carex supina* Willd. ssp. *spaniocarpa* (Steud.) Hult.
271. *Carex concinna* R. Br.
272. *Carex glacialis* Mack.
273. *Carex rariflora* (Wahl.) J. E. Smith.

PLATE XIII

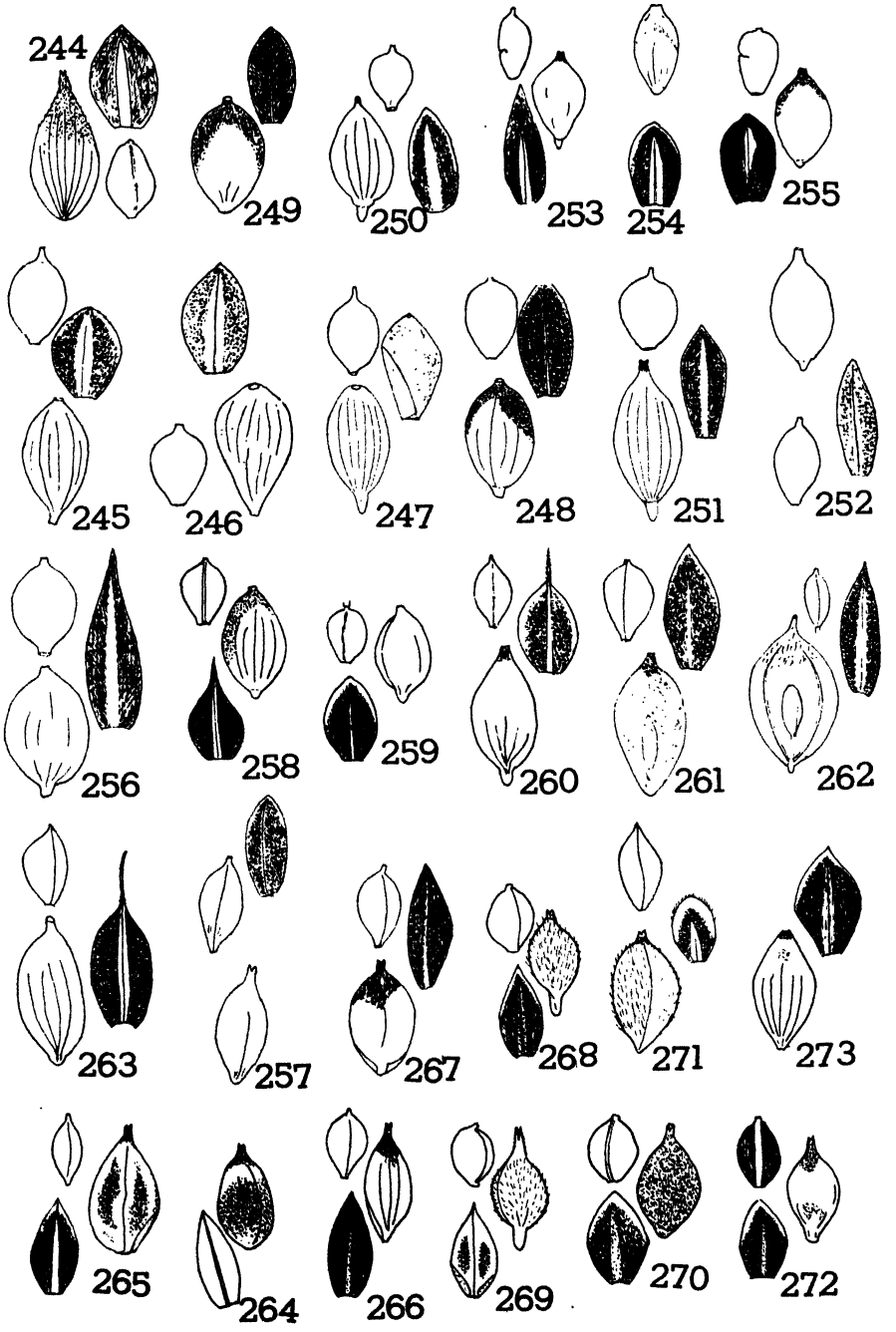

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PLATE XIV.

Fig.

274. *Carex pluriflora* Hult. All the illustrations of Carex show a scale, perigynium and usually the achene.
275. *Carex limosa* L.
276. *Carex magellanica* Lam.
277. *Carex livida* (Wahl.) Willd.
278. *Carex vaginata* Tausch.
279. *Carex atrofusca* Schk.
280. *Carex misandra* R. Br.
281. *Carex capillaris* L.
282. *Carex viridula* Michx.
283. *Carex rostrata* Stokes.
284. *Carex rotundata* Wahl.
285. *Carex rhyncophysa* C. A. Mey.
286. *Carex physocarpa* Presl.
287. *Carex membranacea* Hook.
288. *Lysichitun americanum* Hult. & St. J. Leaf and inflorescence.
289. *Calla palustris* L. Leaf and fruiting spathe.
290. *Lemna trisulca* L. Group of fronds.
291. *Lemna minor* L. Floating frond.
292. *Juncus filiformis* L. All illustrations of Juncus show the capsule inclosed in the perianth with basal bracts when present and the seed.
293. *Juncus drummondii* E. Mey.
294. *Juncus effusus* L.
295. *Juncus arcticus* Willd.
296. *Juncus balticus* Willd. ssp. *sitchensis* (Engelm.) Hult.
297. *Juncus ensifolius* Wiks.
298. *Juncus bufonius* L.
299. *Juncus macer* S. F. Gray.
300. *Juncus stygius* L. ssp. *americanus* (Buch.) Hult.
301. *Juncus biglumis* L.
302. *Juncus triglumis* L.
303. *Juncus mertensianus* Bong.

PLATE XIV.

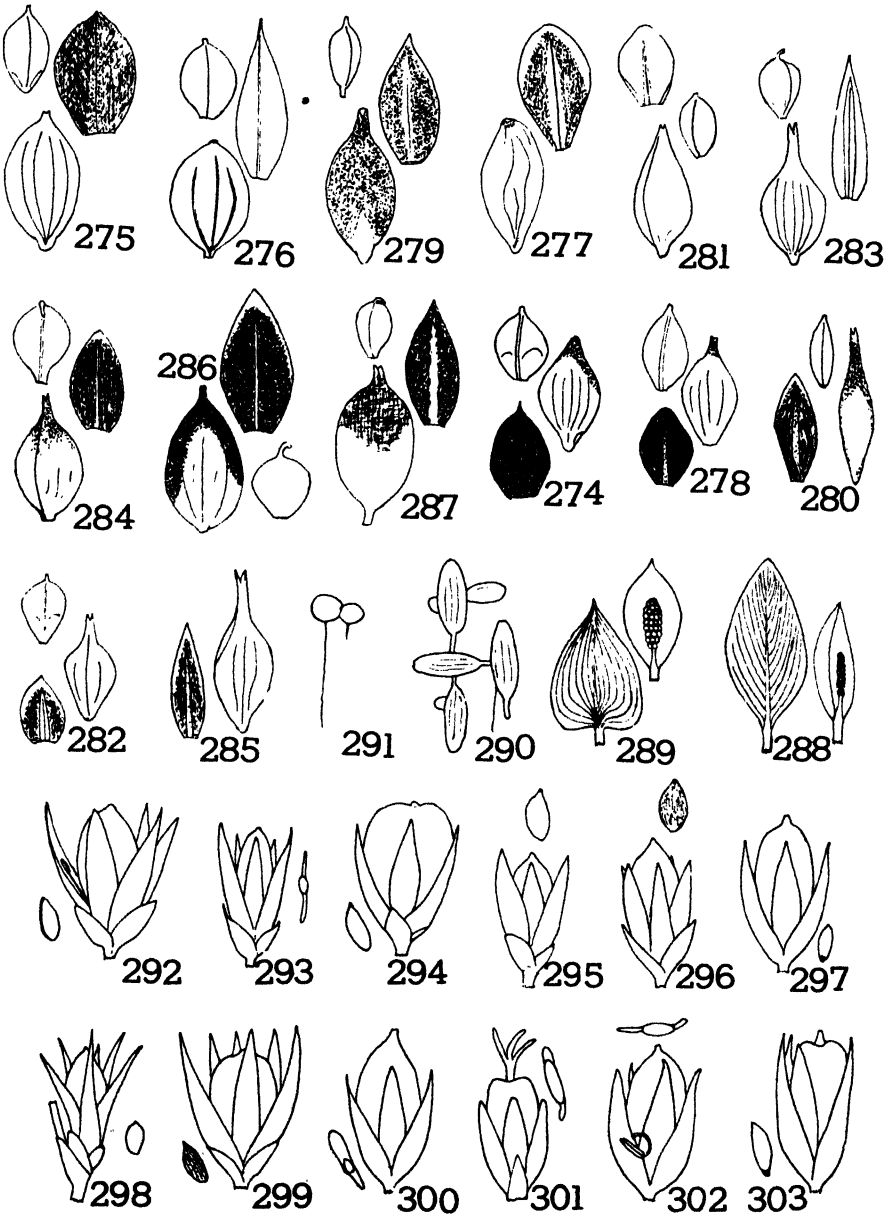

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PLATE XV.

Fig.

304. *Juncus alpinus* Vill. ssp. *nodulosus* (Wahl.) Lindm. All illustrations of Juncus and Luzula show capsule inclosed in the perianth with bractlets and a seed.
305. *Juncus nodosus* L.
306. *Juncus falcatus* E. Mey. ssp. *sitchensis* (Buch.) Hult.
307. *Juncus castaneus* J. E. Smith.
308. *Luzula rufescens* Fisch.
309. *Luzula wahlenbergii* Rupr.
310. *Luzula parviflora* (Ehrh.) Desv.
311. *Luzula spicata* (L.) DC.
312. *Luzula arcuata* Wahl.
313. *Luzula hyperborea* R. Br.
314. *Luzula nivalis* (Laest.) Beurl.
315. *Luzula multiflora* (Retz.) Lej.
316. *Tofieldia occidentalis* S. Wats. Flower and leaf.
317. *Tofieldia pusilla* (Michx.) Pers. Flower and leaf.
318. *Tofieldia coccinea* Richards. Flower and leaf.
319. *Veratrum eschscholtzii* A. Gray. Leaves, flower and capsule.
320. *Zygadenus elegans* Pursh. Flower, petal and capsule.
321. *Allium sibiricum* L. Flower and leaf.
322. *Allium victoralis* L. ssp. *platyphyllum* Hult. Leaf.
323. *Lloydia serotina* (L.) Wats. Leaf, capsule and flower.
324. *Fritillaria camtschatcensis* (L.) Ker. Bulb, flower and leaf.
325. *Clintonia uniflora* (Schult.) Kunth. Flower, leaf and berry.
326. *Maianthemum dilitatum* (Wood) Nels. & Macb. Leaf and flower.
327. *Smilicina racemosa* (L.) Desf. Flower and leaf.
328. *Smilicina stellata* (L.) Desf. Flower and leaf.
329. *Kruhsea streptopoides* (Ledeb.) Kearney. Flower, leaf and petal.
330. *Streptopus amplexifolius* (L.) DC. Leaf, flower and petal.
331. *Streptopus roseus* Michx. ssp. *curvipes* (Vail) Hult. Leaf, petal and flower.

PLATE XV.

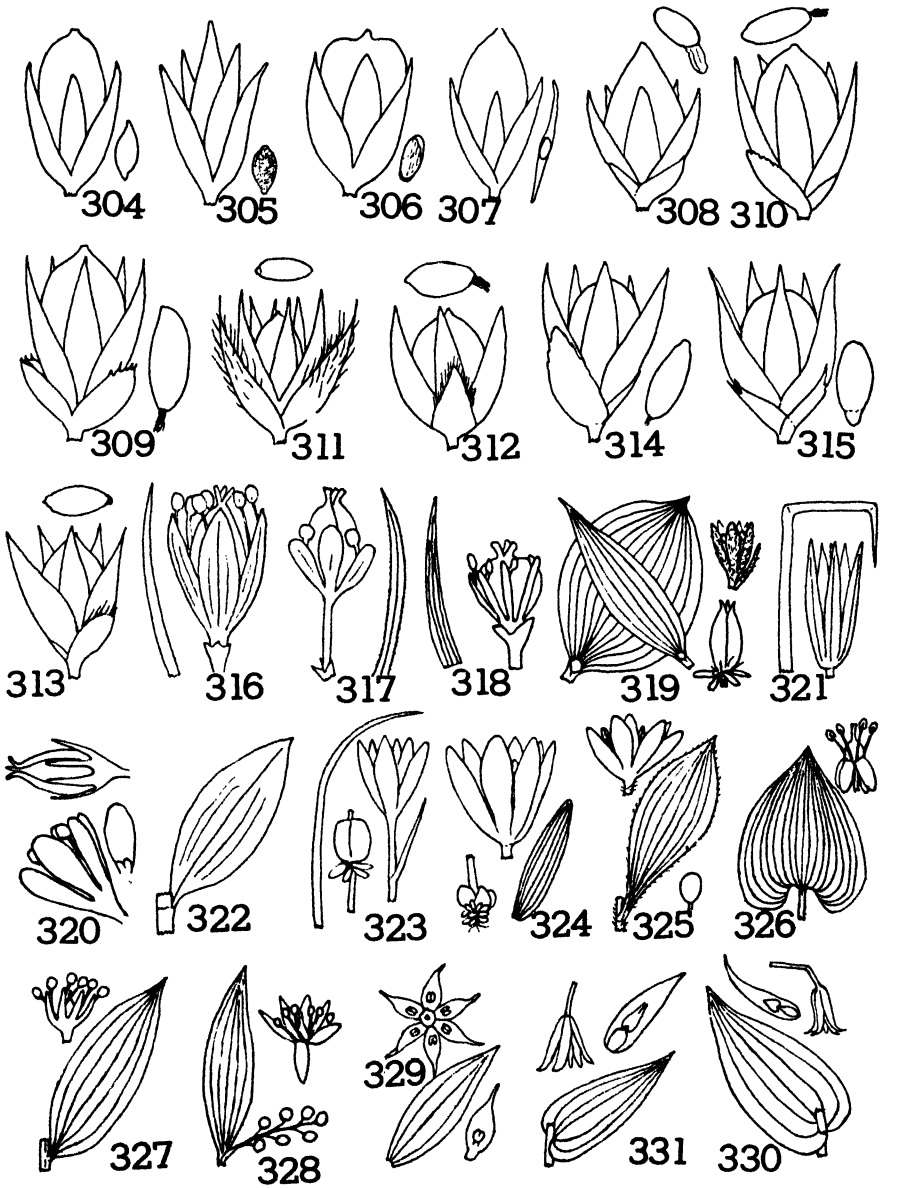
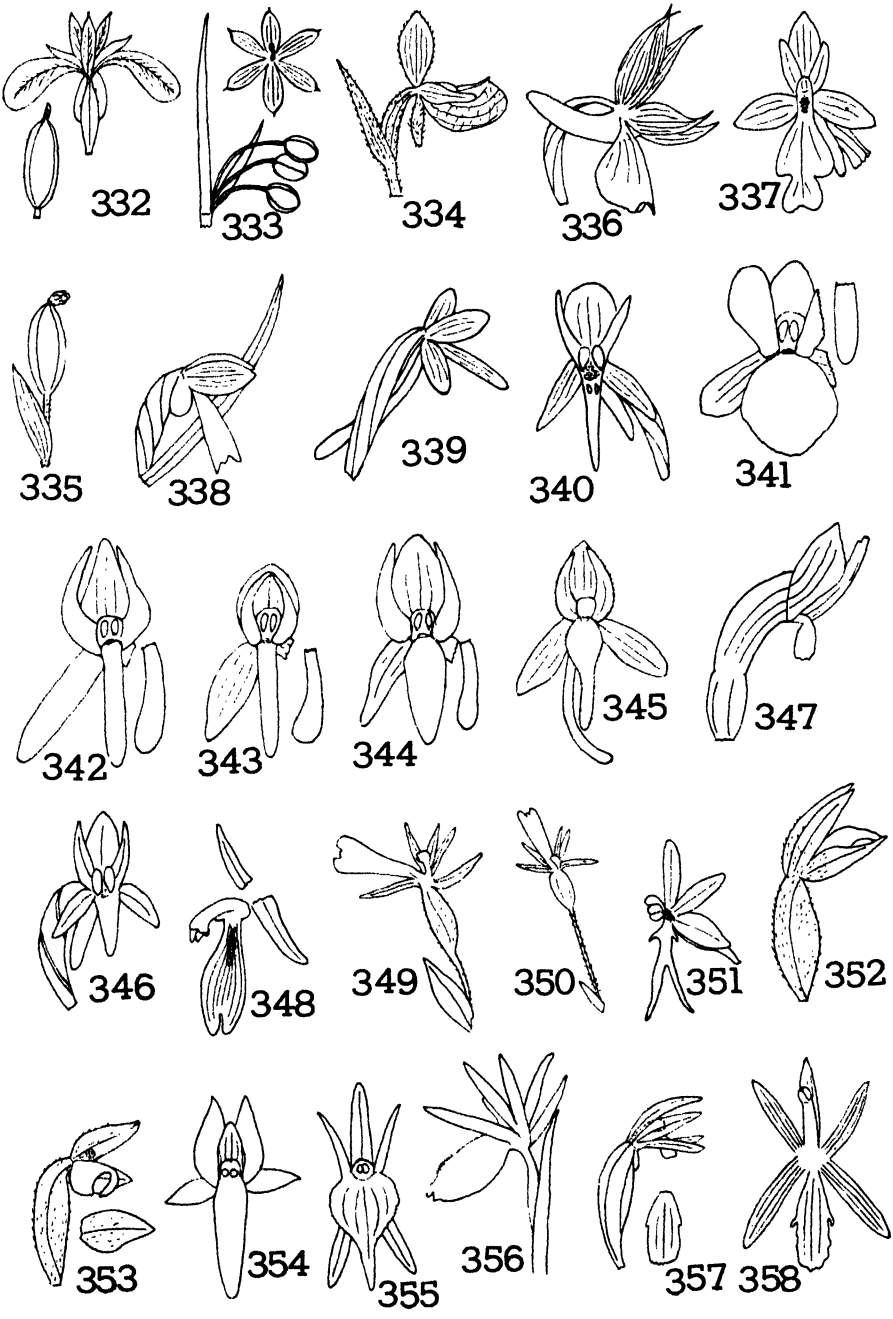

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PLATE XVI.

Fig.

332. *Iris setosa* Pall. Flower and capsule.
333. *Sisyrinchium littorale* Greene. Flower and cluster of capsules with bracts.
334. *Cypripedium guttatum* Sw. Side view of flower.
335. *Cypripedium passerinum* Richards. Capsule.
336. *Orchis aristata* Fisch. Side view of flower.
337. *Orchis rotundifolia* Pursh. Front view of flower.
338. *Coeloglossum viride* (L.) Hartm. Flower and bract.
339. *Lysias orbiculata* (Pursh) Rydb. Side view of flower, petals hidden.
340. *Lysiella obtusata* (Pursh.) Rydb. Front view of flower.
341. *Limnorchis chorisiana* (Cham.) J. P. Anderson. Front view of flower and spur.
342. *Limnorchis convallariaefolia* (Fisch.) Rydb. Front view of flower and detached spur.
343. *Limnorchis stricta* (Lindl.) Rydb. Front view of flower with detached spur.
344. *Limnorchis hyperborea* (L.) Rydb. Flower, as above.
345. *Limnorchis dilitata* (Pursh.) Rydb. var. *leucostachys* (Lindl.) Ames. Front view of flower.
346. *Piperia unalaschensis* (Spreng.) Rydb. Flower.
347. *Spiranthes romanzoffiana* Cham. & Schleicht. Side view of flower.
348. *Listera borealis* Morong. Floral parts.
349. *Listera convallarioides* (Sw.) Torr. Flower with bract.
350. *Listera caurina* Piper. Flower with bract.
351. *Listera cordata* (L.) R. Br. Parts of flower in position.
352. *Peramium decipiens* (Hook.) Piper. Side view of flower.
353. *Peramium repens* (L.) Salisb. var. *ophioides* Fern. Side view of flower with one sepal removed.
354. *Malaxis paludosa* (L.) Sw. Front view of flower.
355. *Malaxis monophylla* (L.) Sw. Front view of flower.
356. *Calypso bulbosa* (L.) Rchb. Side view of flower.
357. *Corallorrhiza trifida* Chatelin. Flower and lip.
358. *Corallorrhiza mertensiana* Bong. Floral parts.

PLATE XVI.


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OBSERVATIONS ON OVIPOSITION AND ADULT SURVIVAL OF SOME GRASSHOPPERS OF ECONOMIC IMPORTANCE[1]

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Although a considerable amount of general information concerning some grasshoppers has accumulated during the past 50 years, certain aspects of grasshopper biology have been entirely neglected, or have received attention only with small samples. This scarcity of information is particularly marked in regard to factors of economic importance such as the average number of egg-pods deposited per female; the average number of eggs produced; and the span of days the adult female has for her ovipositional activity.

Riley (1891) reviewed the early literature and concluded with the sentence, "The number of eggs produced by a well-developed locust will range from 100 to 150, if we consider species generally." Riley early realized that egg production by grasshoppers was sharply affected by weather conditions; and it was his opinion that the laying season normally extended from 6 to 8 weeks, during which an average of 3 egg masses were formed at intervals of about 2 weeks between pods.

While experimenting in 1924 with *Melanoplus mexicanus* (Sauss.), Parker (1930) found that one female deposited 15 pods, and that the average for 30 females was 8.8 pods. Egg masses were deposited at average intervals of 4 to 5 days. The number of eggs per pod varied from 12 to 36 but averaged no higher for females laying a few pods than for those laying many.

In other experiments, Parker (1930) found that ten females of *M. mexicanus* (Sauss.) averaged 5 pods; *M. bivittatus* (Say), 3.6 pods; and the maximum number of pods by a single female was 15 for *M. mexicanus* and 4 for *M. bivittatus*. Later, in a popular bulletin, Parker (1939) said: "The number of eggs in each pod varies with the species. The egg pods of the clear-winged and migratory grasshoppers usually contain from 15 to 20 eggs, while those of the differential and two-striped grasshoppers have from 50 to 75. The number of pods laid by each female varies according to the species, the food supply, and weather conditions. A female migratory grasshopper held in a cage laid 21 pods. The two-striped grasshopper has been known to lay 12 pods, and the differential grasshopper 8."

Sanderson (1939), working with *Melanoplus differentialis* (Thom.), found that 17 females fed entirely upon soybeans produced an average of

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304.6 eggs. The maximum number of eggs deposited by a single female was 645, and each of 4 individuals deposited more than 500 eggs. The 17 females laid 47 pods containing 5,262 eggs, an average of 111.95 eggs per pod. Five females fed entirely on wheat produced an average of 208.6 eggs each. The only female that reached maturity on cotton deposited a single pod containing 73 eggs.

Shotwell (1941) summarized his information by writing, "In one observation 40 females of *M. differentialis* deposited between 12,000 and 13,000 eggs, or an average of over 300 eggs per female. Five pairs of *M. bivittatus* caged separately averaged 2 pods and 127 eggs per female. By actual count, the greatest number of eggs laid by any female of *M. bivittatus* in one pod was 104. The largest number of pods obtained from 1 female was 4."

The foregoing records present considerable information on the reproductive potential of certain grasshoppers, but the data, in some cases, are based on small samples, and have been obtained by methods which are not basically similar or comparable. There is, therefore, need for additional research to reach a better comprehension of the normal or average fecundity of the major grasshoppers causing heaviest losses to cultivated crops.

The present paper contains data from efforts to determine normal egg-laying records of some common species, especially those which have been of economic importance in Iowa, along with observations on some of the factors which may influence experiments of this kind. The maintenance technique used in caging and caring for the grasshoppers in some trials was purposely kept as close as possible to conditions which might prevail in the field.

MATERIALS AND METHODS

The grasshoppers used in the experiments were collected each year, beginning in 1938, from heavily infested fields and along roadsides in Crawford, Harrison, Pottawattamie, and Monona counties, all in western Iowa. Since the work was initiated each spring just as the 'hoppers began to attain the adult stage, collections consisted mostly of individuals in the last nymphal stage, and a few newly emerged imagoes. In order to reduce injury of handling to a minimum, collections were made, whenever possible, in the early evening after the 'hoppers had crawled up on tall vegetation to "roost" for the night. The 'hoppers were transported immediately, in small screened cages, with food, to the insectary at Ames and there confined in screened, cubical cages of 6-foot dimensions for a few days so as to eliminate injured individuals and to permit more nymphs to reach the adult state. In this way it was possible to collect at random from stock cages newly emerged, healthy, unmated, male and female adults for transfer to the smaller experimental cages. Here, again, individuals which died before the beginning of oviposition were replaced by unmated specimens of the same sex from one of the two stock cages containing either segregated males or females.

Every effort was made to secure an heterogeneous population by random sampling in the field and from the stock cages. Individuals injured in field collections, transportation, and handling were the only specimens eliminated. No attempts were made to select for size; but any obviously deformed specimens, and those without a full complement of legs were discarded. No changes or additions were made in the experimental cage after egg-laying had once started. Dead specimens were removed daily. An abundance of succulent food, as described below, was available for feeding and resting purposes at all times in the cages.

Two types of cages were used in the experimental work. The smaller cage was 1 foot square at the base, 2 feet high, and equipped with a 6-inch deep, removable soil tray. The front and back were sliding glass panels; the other two sides and the top were covered with 14-mesh wire screen. About 5 inches of sifted, steam-sterilized, moist, sandy loam were placed in each tray and firmed down with a small wooden block. The soil was removed and sifted at weekly intervals in order to determine the number of egg-pods and eggs laid each week. Some of these smaller cages were kept under roof in a screenhouse; others were maintained outdoors in full sunlight. Five pairs of grasshoppers of the same species were caged in each small cage.

The larger experimental cage enclosed approximately 10 times (10 sq. ft.) as much surface as the small cage at the base, was 3 feet high, and was covered on the top and sides, including the door, with 14-mesh wire screen. All of these cages were securely staked outdoors on an unshaded plot of bluegrass sod. Fifty pairs of grasshoppers of a species were confined in each of the larger cages. These cages were not taken up until after all adults had died, when the soil was sifted to collect the egg-pods for counting.

All experimental cages, both large and small, for one species of grasshopper were always started on the same day.

During the summer of 1938, the small cages were placed on sand benches in a roofed screenhouse. As the season progressed, it appeared that the grasshoppers confined in the cages on the extreme south end of the benches were living a little longer and were depositing a few more eggs than those farther inside and not receiving any direct rays of the sun. As this difference was very slight, and the sample small, parallel experiments were planned for the next summer, so as to determine if there were any significant differences in results from grasshoppers confined in shaded cages and those kept outdoors, fully exposed to direct sunlight, higher temperature, freer wind currents, and other weather conditions.

Only the small type of cages was used in the parallel experiments. Sets of 20 cages were operated in the screenhouse again in 1939 and 1940, while other sets of 20 cages were placed in the open on lawn sod so as to have maximal exposure to outdoor environmental surroundings. Feeding and care of the two sets of cages in the parallel experiments were as nearly identical as it was possible to operate them. Whatever ecological differ-

ences existed in these parallel experiments were the results of the different exposures in the locations of the two sets of cages.

FEEDING TECHNIQUE

Special precautions were taken at each feeding to put a similar and adequate supply of mixed succulent food in every cage during the entire course of the experimental work. Fresh food was provided twice daily for each cage. The items of diet were identical for all cages at each feeding. An excess of food was provided each time so that the grasshoppers would never be without feed. The first feeding took place between 8 and 10 a. m. and the second between 3 and 5 p. m.

Young corn (*Zea mays*) and a legume [either alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), soy bean (*Glycine* sp.), or sweet clover (*Melilotus* sp.)] were the staple items of diet at every feeding. In addition, two or three other cultivated or wild plants in smaller quantities were always placed at each feeding in every cage. The miscellaneous plants consisted of an assortment of wild vegetation, garden vegetables and forage plants frequently eaten by grasshoppers in the field or in gardens. Hemp (*Cannabis sativa*), wild sunflower (*Helianthus* sp.), wild mustard (*Brassica* sp.), ragweed (*Ambrosia* sp.), wild lettuce (*Lactuca* sp.), various grasses (*Alopecurus pratensis, Digitaria sanguinalis,* and *Agropyron repens*), and garden crops such as lettuce (*Lactuca* sp.), cabbage (*Brassica oleracea*), onion (*Allium* sp.), potato vines (*Solanum tuberosum*), beet greens (*Beta* sp.), and other cultivated plants, when available in sufficient quantities to supply all the cages, were used to supplement the corn and legume staples of the mixed diet. All cut plants were always inserted in a jar of fresh water to keep the plants succulent until the next feeding. Since the legume and supplementary items were varied from feeding to feeding, a considerable choice of food was offered every day to the 'hoppers. Occasionally 4-inch pots of newly sprouted oats (*Avena sativa*) or wheat (*Triticum aestivum*) about 5 to 6 inches high, were placed in each cage. Such pots of growth could be watered and left in the cage between egg-collecting intervals until "grazed" to the soil. However, such feeding of potted plants entailed extra work in careful examination of the potted soil to recover any eggs which might have been laid in them.

Corn was planted at intervals during the spring and summer for feeding purposes. Weeds and most of the other wild or cultivated plants varied in size and availability with their seasonal development during the summer. As soon as available, fresh ears of sweet or field corn in the milky stage were put in each cage two or three times a week. Part of the husk on each ear was stripped back to expose the silk and kernels.

At the same time that these experiments on mixed diet were in operation, other observations on the effects of a diet of single food plants were being made. Results from these restricted diet tests are reported by Tauber, Drake and Decker (1945) in a separate paper.

SPECIES STUDIED

The experiments were confined to the three most important economic species (Drake and Decker, 1938) of grasshoppers occurring in Iowa: The two-striped grasshopper [*Melanoplus bivittatus* (Say)]; the differential grasshopper [*M. differentialis* (Thom.)]; and the lesser migratory grasshopper [*M. mexicanus* (Sauss.)]. These three pests have been responsible for 90 to 95 per cent of the total grasshopper damage inflicted on Iowa crops (Drake and Tate, 1938) since early settlers began farming.

Methods of collecting, handling, caging, and feeding were the same for all three species.

ADULT LONGEVITY

The time of hatching, the rate of nymphal development, and the subsequent appearance of adults are influenced by the earliness or lateness of the season and many other factors, and thus vary from year to year. Examples of two of these variables, average daily temperature and monthly rainfall, are shown in Table 1.

TABLE 1

METEOROLOGICAL DATA FOR AMES, IOWA

Month	Normal	1938	1939	1940	1941	1942
	Average Daily Temperature					
April	48.8	50.3	47.8	47.5	53.8	54.8
May	60.2	59.5	66.4	58.4	66.0	59.3
June	69.6	69.2	71.4	71.3	70.0	69.7
July	74.7	76.5	76.2	76.7	75.1	74.3
August	72.2	75.7	70.7	70.7	75.1	72.2
September	63.9	66.8	69.3	65.8	66.6	61.4
	Precipitation for Month					
April	2.70	3.66	2.07	3.22	2.50	1.06
May	4.03	5.45	2.07	2.07	3.26	4.70
June	4.65	4.67	5.32	3.56	6.20	5.93
July	3.67	4.24	3.15	4.57	2.24	4.89
August	3.58	3.82	4.72	6.44	1.94	3.17
September	3.83	5.67	0.82	0.94	7.74	4.13

Over most of Iowa, adults of the two-striped grasshopper and the first generation of the lesser migratory grasshopper begin to appear about the middle of June and are practically all dead by early September. The adult life span in the field appears to average between 6 and 7 weeks, with a maximum of about 100 days. The differential grasshopper does not become adult until early July and is usually present in the fields in considerable numbers until late September or early October. By that time only a few stragglers of the two-striped species, and large numbers of both nymphs and adults of the second generation of the lesser migratory

grasshopper are then present. A few individuals of all three species remain until cold weather begins to decimate their numbers. Field observations indicate that the females of all three species tend to live longer than the males. Records from caged grasshoppers confirm this difference in adult life spans.

An example of summarized data from one season's work with *M. bivitattus* is shown in Figure 1. In that year (1940) all males had died by the end of the sixth week after the onset of egg-laying; but some females under identical conditions survived until the ninth week.

In the present paper the mortality which occurred among females between emergence and just prior to egg deposition is not given because any individuals were replaced which died in the cages before oviposition began. Unless signs of parasitism were plainly evident it was impossible to separate those which died of a natural cause from those that died from some other causes. Figure 1 also shows a typical year's results in deaths associated with nematode parasitism. This problem of roundworm infection will be covered in a subsequent paper by Tauber. No replacements were made in any of the cages of a series of experiments after egg-laying had once started in that series.

Curves showing the yearly mortality of females of the three species of grasshoppers, confined in the large outdoor cages, are given in Figures

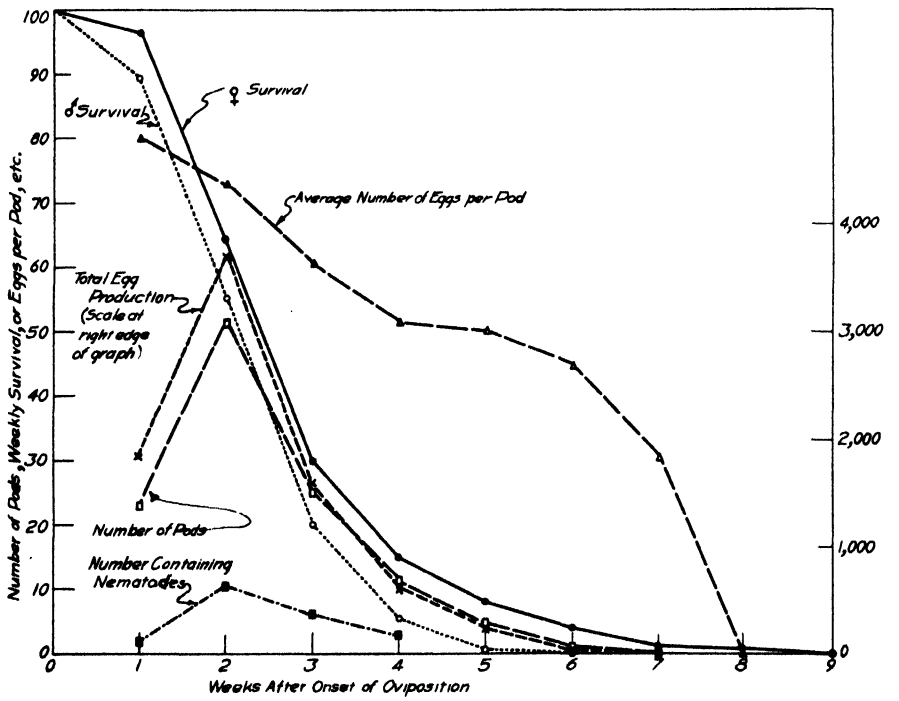


Fig. 1. Curves summarizing a typical year's (1940) observations of *Melanoplus bivittatus* on a mixed diet.

2, 3, and 4. A compilation of 3 years' data (1939-41) is presented by the averages in Figure 5. The trends of the curves were influenced to some extent by variations in local weather conditions. (Compare weather data of Table 1 with the above mortality curves.) In general, however, the

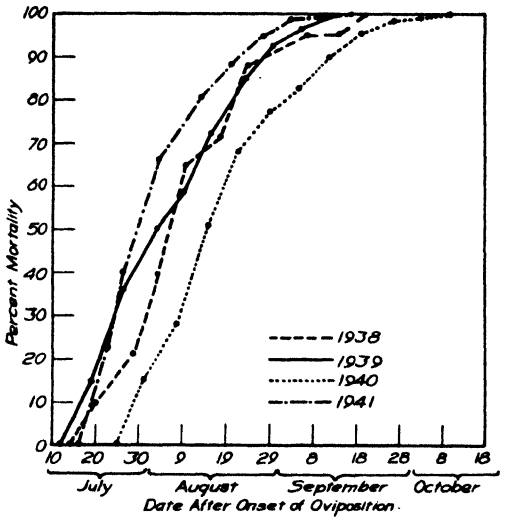


Fig. 2. Curves showing adult female mortality of *Melanoplus bivittatus* during four summers.

data are expressed in typical sigmoid curves with nearly straight lines within a range of 20 to 80 per cent mortality. Approximately 25 per cent of the females in all the experiments died within 3 weeks after the first

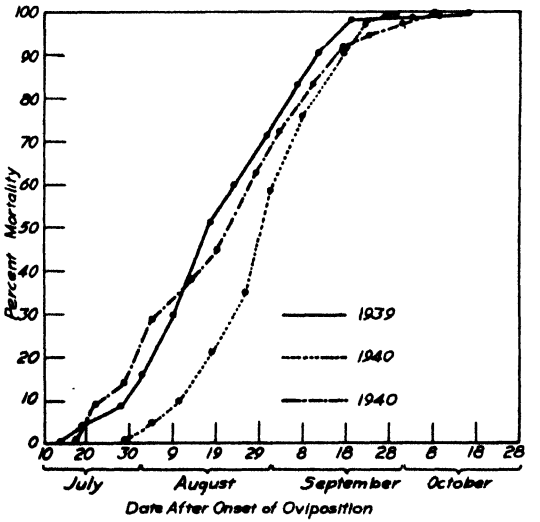


Fig. 3. Curves showing adult female mortality of *Melanoplus mexicanus* during three summers.

observed oviposition, 50 per cent in 5 weeks, and 75 per cent in 7 weeks. Some of the individuals of the remaining population of 25 per cent lived for a much longer time and thus decreased the slope at the top of the curve.

Mortality curves of grasshoppers provide information needed for tim-

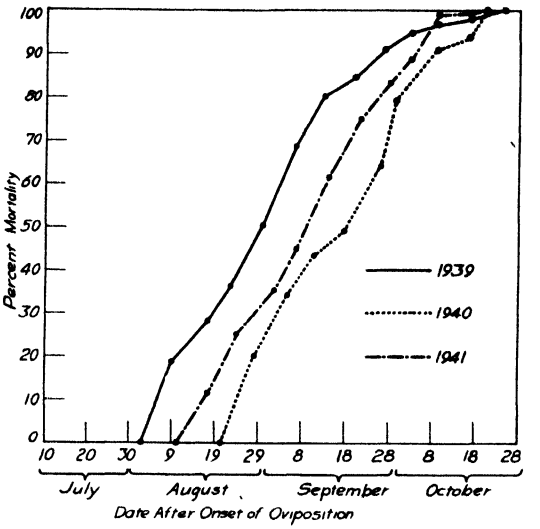


Fig. 4. Curves showing adult female mortality of *Melanoplus differentialis* during three summers.

ing and evaluating adult population surveys in the fall. Figures 2, 3, and 4 show how such curves may be altered from year to year by earliness or lateness of the season and other climatic factors. Correct evaluation of

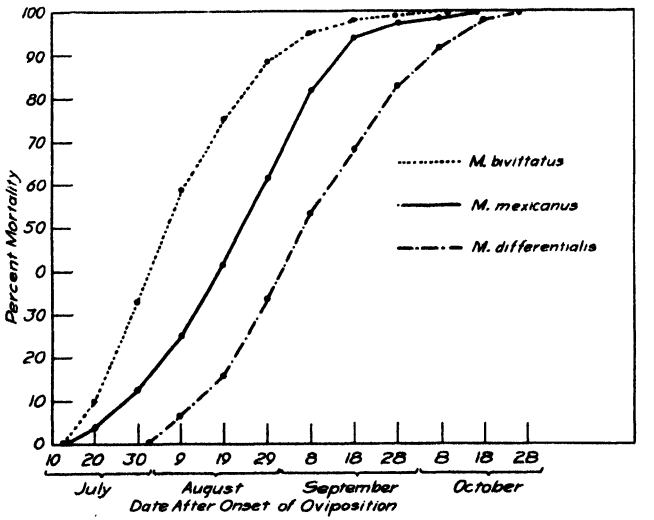


Fig. 5. Three year averages (1939–41) of adult female mortality of *Melanoplus bivittatus*, *M. mexicanus*, and *M. differentialis*.

a population of one or several species will depend upon the field scout's knowledge of the mortality trend and the amount of egg-laying which has already occurred in the area for each species. Shotwell (1938) allows in part for this factor by setting up separate evaluation tables for collections made early and late in the season. Many state workers have found it necessary to make still other adjustments for local conditions. The importance of differentiating between species in field surveys has not always received adequate emphasis. If a survey comes at the peak of activity by *M. differentialis* in populations involving other species, as is often the case, about 75 per cent of *M. bivittatus* and 50 per cent of *M. mexicanus* females will not be accounted for because they have already died of old age and thus passed entirely out of the picture. It should be pointed out that the adults of the three species used in the present experiments were collected early in the season and represent largely that part of the population present at the beginning of adult emergence; hence, the complete mortality curve, based on field observations for an entire season of these species, would be represented by curves extending over a longer period of time, perhaps 2 weeks or a little longer.

When the mortality curves for female grasshoppers kept in small cages inside the screened insectary are compared with similar curves from the same type of cages out-of-doors, it is apparent that sunlight and other climatological factors had an important bearing on the length of adult life. (See fig. 6). The grasshoppers confined in cages in the screened insectary invariably died sooner than those confined in similar cages outdoors and thus more fully exposed to the weather. The average length of life of *M. bivittatus* females was 21.48 days in the small cages in the screened insectary; 29.04 days in the same type of cage out-of-doors; 28.37 in the large cages out-of-doors. These averages are based on 100 pairs of grass-

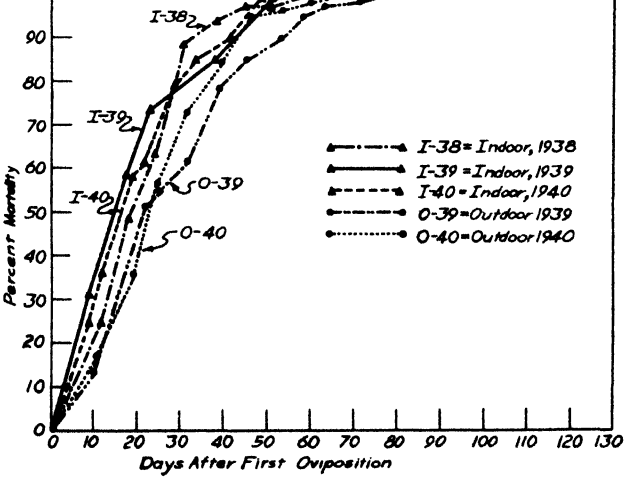


Fig. 6. Comparison of adult female mortality of *Melanoplus bivittatus* in cages indoors and outdoors.

hoppers with each variable, repeated in parallel for 2 years. At the 50 per cent mortality point on the curves from the parallel small cage experiments, a full week of difference in survival was noted. Such results make it clear that investigators should take special care in expressing clearly the exact environmental conditions of their experiments.

OVIPOSITION

Dates of the first observed oviposition by the three species of grasshoppers in outdoor cages are given in Table 2 for the years 1939-41. These dates were within a few days of the initial egg-laying noted for the same species in the field, in the respective years.

TABLE 2

DATES OF FIRST OBSERVED OVIPOSITION IN FIELD CAGES AT AMES, IOWA, BY SPECIES AND YEARS

Species	Year		
	1939	1940	1941
M. mexicanus	July 13	July 29	July 16
M. bivittatus	July 12	July 25	July 16
M. differentialis	August 2	August 21	August 10

COMPARISON OF EGG-LAYING IN SHADED OR FULLY-LIGHTED CAGES:

The experimental results with *M. bivittatus* in 1939 showed that females confined in the small cages in the roofed screen-sided insectary laid an average of 101 eggs, whereas those in the large outdoor cages averaged 147 eggs per female. As stated previously, a slight difference was noted in the number of eggs obtained per female in some of the cages in the screened insectary; those on the south side, exposed to a limited amount of sunlight, contained a few more eggs.

In 1939 and 1940, when parallel experiments were set up, the results showed a marked difference between the small cages located indoors and those outdoors. When the data for *M. bivittatus* in the small cages indoors and similar cages outdoors are compared, the ratio in egg deposits is approximately two to one in favor of the cages placed in the open outdoors. (See Table 3 and fig. 6.)

Similarly, when parallel experiments of small cages containing *M. differentialis* were set up in the same manner, an even greater difference in results was noted, with approximately a five to two ratio in favor of the fully exposed cages. The difference may be partially accounted for in the longer adult life span of the species when caged outdoors.

COMPARISON OF DATA FROM SMALL AND LARGE OUTDOOR CAGES:

In comparing other data (Table 4) between the small and large cages, both located in the open outdoors, the number of pods per female, as well as the number of eggs laid per female day, are lower in the larger cages. Perhaps the disturbances caused by the larger number of grasshoppers

TABLE 3

AVERAGE NUMBER OF PODS AND EGGS PER FEMALE FROM 20 SMALL CAGES WITH 5 PAIRS OF GRASSHOPPERS EACH, BY SPECIES AND YEARS

	M. bivittatus					*M. differentialis*		
	Cages Indoors			Cages Outdoors		Cages Indoors		Cages Outdoors
	1938	1939	1940	1939	1940	1938	1939	1939
Pods per female:								
Maximum in one cage	2.4	2.4	1.6	5.0	3.6	1.8	1.6	2.8
Minimum in one cage	0.6	0.4	0.8	1.0	1.4	.0	.0	0.8
Average for all cages	1.2	1.2	1.2	2.6	2.4	1.0	0.6	1.8
Eggs per female:								
Maximum in one cage	212.6	169.2	105.8	322.0	253.8	218.0	164.8	249.6
Minimum in one cage	37.8	25.0	62.2	89.2	91.0	.0	.0	66.8
Average for all cages	101.2	90.7	81.5	183.7	163.6	108.4	59.6	146.2

TABLE 4

INFLUENCE OF SIZE AND LOCATION OF CAGES ON OVIPOSITION BY 100 PAIRS OF *M. bivittatus* AND *M. differentialis*

	M. bivittatus			*M. differentialis*
	1939	1940	Av. '39 & '40	1939
Counts From 20 Small Cages Indoors				
Eggs	9,075	8,155	8,615	5,956
Pods	122	120	121	65
Total ♀ days	2,222	2,074	2,148	3,556
Pods per ♀	1.2	1.2	1.2	0.6
Eggs per pod	74.4	67.9	71.2	91.2
Eggs per ♀	90.7	81.5	86.1	59.6
Eggs per ♀ day	4.1	3.9	4.0	1.7
Counts From 20 Small Cages Outdoors				
Eggs	18,374	16,363	17,368	14,620
Pods	257	240	248	184
Total ♀ days	3,002	2,806	2,904	4,632
Pods per ♀	2.6	2.4	2.5	1.8
Eggs per pod	71.5	68.2	69.9	79.4
Eggs per ♀	183.7	163.6	173.7	146.2
Eggs per ♀ day	6.1	5.8	6.0	3.2
Counts From 2 Large Cages Outdoors				
Eggs	12,761	13,317	13,039	14,025
Pods	177	201	189	153
Total ♀ days	2,835	2,843	2,839	3,960
Pods per ♀	1.8	2.0	1.9	1.5
Eggs per pod	72.1	66.2	69.1	91.7
Eggs per ♀	127.6	133.2	130.4	140.2
Eggs per ♀ day	4.5	4.7	4.6	3.5

TABLE 5

OVIPOSITION RECORDS FOR 100 FEMALES OF EACH SPECIES BY YEARS IN LARGE CAGES OUT OF DOORS

	1938	1939	1940	1941	Total	Yearly Average
M. bivittatus						
Eggs	14,733	12,761	13,317	10,744	51,555	12,888.7
Pods	210	177	201	153	741	185.3
Total ♀ days	3,113	2,835	2,843	2,261	11,052	2,763.0
Pods per ♀	2.1	1.8	2.0	1.5		1.9
Eggs per pod	70.1	72.1	66.2	70.2		69.7
Eggs per ♀	147.3	127.6	133.2	107.4		128.9
Eggs per ♀ day	4.7	4.5	4.7	4.7		4.6
M. differentialis						
Eggs		14,025	11,580	12,761	38,366	12,788.6
Pods		153	138	141	432	144.0
Total ♀ days		3,930	3,620	3,746	11,296	3,765.3
Pods per ♀		1.5	1.4	1.4		1.4
Eggs per pod		91.7	86.1	90.5		88.8
Eggs per ♀		140.2	115.8	127.6		127.9
Eggs per ♀ day		3.5	3.2	3.4		3.4
M. mexicanus						
Eggs		14,122	10,926	10,131	35,179	11,726.3
Pods		703	558	531	1,792	597.3
Total ♀ days		3,685	2,600	3,290	9,575	3,191.7
Pods per ♀		7.0	5.6	5.3		6.0
Eggs per pod		20.1	18.9	19.1		19.6
Eggs per ♀		141.2	109.3	101.3		117.2
Eggs per ♀ day		3.8	4.2	3.8		3.7

jumping, crawling, and laying eggs in the larger cages partially account for the divergences. When large numbers are confined in one cage, movements outside of the cages as well as movements of 'hoppers within cause considerable excitement and disturbance within the cage.

Since 5 pairs of adults were caged in each small cage, and 50 pairs in each large cage, there is no way of knowing how many females died without laying eggs; likewise, it is impossible to determine exactly the number of pods or eggs produced by each female. It is quite certain that some females die without depositing any eggs. In fact, females in some of the small cages of *M. differentialis* in the screenhouse died without depositing a single pod. (See Table 3.) Any attempt to explain such a failure to oviposit is a matter of guesswork. Such absence of oviposition by 5 females in a single group never occurred with *M. bivittatus*, however. In some of the small outdoor cages of *M. bivittatus* each of the 5 females averaged 5 pods (Table 3) containing an average total of 322 eggs. There is no way of checking on this point, but it is highly probable that some of these females laid more than 5 pods to achieve such an average for the group. In other instances it seems fairly evident that a few females probably laid no eggs.

Although the seasonal averages for the number of eggs per pod were rather constant for a species, some variations throughout any one season were observed. When the oviposition data are segregated by weeks, the first pods laid are the largest and contain the greatest number of eggs. Each week thereafter the size of the pods tends to become smaller as the season progresses. It was also evident that not all of the females deposited an egg mass during the first weeks of recorded egg-laying. After the first sifting for egg pods, it was, of course, impossible to differentiate between the first masses and those laid later. Since some females in the outdoor cages lived longer and continued to lay after those in the indoor cages were dead, the average number of eggs per pod from outdoor specimens is smaller, but the number of eggs laid per female is larger (Tables 6 and 7).

TABLE 6

AVERAGE NUMBER OF EGGS PER POD BY WEEKS THROUGHOUT THE OVIPOSITION PERIOD FOR *Melanoplus bivittatus*

	Cages Indoors				Cages Outdoors		
Weekly Intervals	1938	1939	1940	Average	1939	1940	Average
1st	80.6	75.1	80.0	78.6	78.6	71.3	74.9
2nd	81.9	79.1	72.9	78.0	81.8	74.1	77.0
3rd	75.8	78.3	61.2	71.8	72.7	66.2	69.4
4th	75 4	62.4	52.2	63.3	61.1	61.6	61.3
5th	58.2	58.0	50.2	55.5	65.6	60.1	62.8
6th	64.0	66.7	44.5	58.4	58.8	62.9	60.8
7th	52.0	51.5	31.0	44.8	65.4	58.8	62.1
8th					56.0	56.5	56.2
9th					52.0		52.0
10th					49.3		49.3
Average	77.23	74.4	67.95		71.5	68.2	

In the large outdoor cages, which were operated for 3 consecutive years (1939-41), the results are remarkably constant from year to year (Table 5). The data on pods per female, number of eggs per pod, and eggs per female-day seem to fall within the limits of expected variation. The total number of female-days and the average length of life again were the variables of most importance in affecting egg production.

Number of eggs per pod: The complete egg-laying records are summarized in the foregoing tables. During the 3-year period (1939-1941, inclusive) 300 females of *M. differentialis* deposited a total of 432 egg-pods with 38,366 eggs; the largest pod contained 153 eggs; the smallest, 10 eggs; and the average was 88.8 eggs per pod. During 4 years (1938-1941) 400 females of *M. bivittatus* deposited 741 pods containing 51,555 eggs; the largest pod contained 135 eggs; the smallest, 10 eggs; and the average, 69.7 eggs per pod. Three hundred *M. mexicanus* females (1939-1941) deposited 1,792 pods with 35,179 eggs; the largest pod contained 39 eggs; the smallest, 6 eggs; and the average was 19.6 eggs per pod.

TABLE 7

AVERAGE NUMBER OF EGGS PER POD BY WEEKS THROUGHOUT THE OVIPOSITION PERIOD FOR *Melanoplus differentialis*

Weekly Intervals	Cages Indoors			Cages Outdoors
	1938	1939	Average	1939
1st	162	120	141	106
2nd	124.3	104.5	114.2	79.9
3rd	106.7	100.4	103.6	93.9
4th	100	88.1	94	85.3
5th	104.5	90.6	97.5	71.5
6th	106.7	89	97.8	69.2
7th	95	81	88	69.3
8th		66	66	62.8
9th		51	51	68.5
10th				63
Average	108.5	91.2		79.4

When grasshoppers are confined during experimental procedures, egg-laying is sometimes interrupted by the activities of other grasshoppers in the cage, or by other causes. No attempt was made to distinguish incomplete pods from other small pods. When such interruptions occur, a few eggs may be scattered about in the cage. After oviposition once began in any series of cages, all dead females were opened when removed from the cage in order to check on the contents of the reproductive system. In only a very few cases were any large eggs found in the ovaries. A sick or parasitized female is usually either prevented from maturing eggs, or manages to live long enough to deposit any mature ova which may be

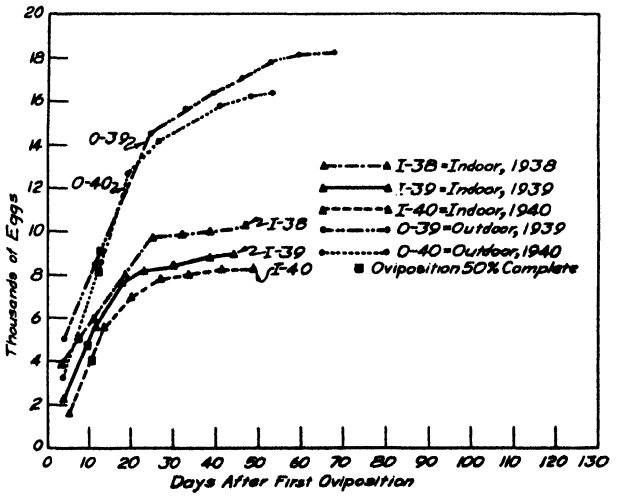


Fig. 7. Curves showing accumulative egg depositions by *Melanoplus bivittatus* in cages indoors and outdoors.

within her. The urge to perpetuate the species is strong. In a few cases dead females were found with the abdomen extended and inserted in the soil, just as in the manner for oviposition.

Average number of eggs per female: The average number of eggs laid per female was 117 for *M. mexicanus*, 128 for *M. differentialis*, and 129 for *M. bivittatus*. The differences in the sizes of the eggs pods of the three species are correlated with the average number of days between pod deposition. Generally speaking, females of *M. mexicanus* laid an egg pod every 4 or 5 days; *M. bivittatus*, every 10 days; and *M. differentialis*, approximately every 2 weeks.

Accumulative egg deposition: Figure 7 charts the accumulative egg deposition by females of *M. bivittatus*. More than the other figures and tables, this illustration shows the difference in egg production by experimental grasshoppers kept in shade or in full sunlight. In all 3 years, the total number of eggs was considerably less indoors. The longer period of productiveness by those specimens outdoors is also obvious. Both indoors and outdoors, however, half of the total egg production was completed within 8-14 days after the onset of oviposition by females in any particular series of cages.

SUMMARY AND CONCLUSIONS

1. These investigations were limited to observations on the egg-production and the life span of adults of the three grasshopper species most destructive to the crops of Iowa: *Melanoplus bivittatus* (Say), *M. differentialis* (Thom.), and *M. mexicanus* (Sauss.).

2. Last instar nymphs and newly emerged imagoes of the three grasshoppers were collected in Harrison, Monona, Crawford, and Pottawattamie counties of western Iowa. Specimens were carefully picked out of the nets and placed in screened cages containing coarse vegetation for feeding and resting purposes, and transported immediately to the insectary at Ames.

3. These stock grasshoppers were confined in large screened cages of 6-foot dimensions on sod in the open insectary yard. Injured and dead grasshoppers were removed daily. An abundant supply of corn, legumes and wild vegetation was placed every morning and afternoon in the cages so that fresh food would be always available for feeding and resting purposes.

4. Two sizes of experimental cages were used: a small type, 12 x 12 x 24 inches; and a large type, 38 x 38 x 36 inches. Five pairs of newly emerged adults were placed in each of the small cages; and 50 pairs in each large cage, covering approximately 10 square feet of sod. An experiment consisted either of a series of 20 small cages (100 pairs of grasshoppers) in comparison with 2 large cages (100 pairs of grasshoppers); or consisted of parallel set-ups in which two series, each of 20 small cages with 100 pairs of 'hoppers, were operated under different conditions. All cages for one experiment were started on the same day. The mixed diet of corn, legumes, other cultivated plants, and weeds was provided twice daily for all cages.

5. During the summer of 1938 the smaller cages were placed on sand benches in the roofed insectary with screened sides, and the larger cages on bluegrass sod in the open yard. The grasshoppers on the south side of the screenhouse tended to live a little longer and deposited a few more eggs than those in similar cages farther inside. Parallel experiments were run in 1939 and 1940 to determine the difference in results from grasshoppers confined in shaded cages and those placed in the open outdoors so as to be exposed to the maximum of direct sunlight, higher temperatures, freer wind currents, and other weather conditions. Feeding and care of the two sets of cages were as nearly identical as it was possible to operate them, so that whatever environmental variables existed were the result of the different locations of the cages.

6. One hundred pairs of *M. bivittatus* in the shaded cages deposited 122 egg pods or a total of 9,075 eggs (4.1 eggs per female day) in 1939 and 120 egg pods and 8,155 eggs (3.9 eggs per female day) in 1940; whereas the hundred pairs in cages placed outdoors in the open deposited 257 egg pods and 18,374 eggs (6.1 eggs per female day) in 1939, and 240 egg pods or 16,363 eggs (5.8 eggs per female day) in 1940. In the shaded cages *M. differentialis* deposited 65 egg pods or 5.956 eggs (1.7 eggs per female day) in 1939; whereas in the unshaded cages in the open, the same number of pairs deposited 184 egg pods or 14,620 eggs (3.2 eggs per female day) in 1940. These differences in egg production show that direct sunlight and other outdoor exposure factors have a marked influence on oviposition by grasshoppers.

7. The first egg pods deposited by a female (all species) are larger than the pods deposited later. In general, females of *M. mexicanus* deposited an egg pod every 4 or 5 days; *M. bivittatus* every 10 days; and *M. differentialis* every 2 weeks. The largest egg-pod from *M. bivittatus* contained 135 eggs; from *M. differentialis* 153 eggs; and from *M. mexicanus*. 39 eggs. The average number of eggs per pod was 69.7 for *M. bivittatus;* 88.8 for *M. differentialis;* and 19.6 for *M. mexicanus.*

8. The average number of eggs per female was 117 for *M. mexicanus;* 128 for *M. differentialis;* and 129 for *M. bivitattus.*

9. In all experiments, both in full light and in the shaded screenhouse, approximately half of the total egg production was completed within 8 to 14 days after the onset of oviposition.

10. The span of adult life of female *M. bivittatus* grasshoppers may be as much as 2½ weeks longer than that for males in the same type of cage. The adult life span of *M. bivittatus* females in the small cages in the screened insectary was 21.48 days indoors and 29.04 days in similar cages out-of-doors; in the large out-of-doors cages the average female life span was 28.37 days.

11. The mortality and egg-deposition curves of the different species provide data needed for timing and evaluating adult population surveys in the fall. The necessity of carefully differentiating species in such surveys is also evident. Prediction of possible grasshopper outbreaks and

estimates for poisoned bait from year to year are based largely upon the adult and egg surveys.

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NOTE

In Response To "The Dissimilation of Glucose by Chaetomium funicola Cke. III"[1]—The following statement is to be found on p. 347 of this communication:

> "The suggestion by Nord et al. (76, 77, 78) that glucose dissimilation by Fusarium sp. need not go by way of phosphorylation has been criticized (65) on the ground that consideration was given only to the phosphorus in the medium rather than that in the mycelium (102), to which also might be added the anaerobic conditions prevailing under the experiment with consequent phosphorus mineralization (67)."

I wish to call attention to the fact that this utterance is inconsistent with the following:

In the papers quoted under 76 and 78, which were published in 1939 and 1937, respectively, data are recorded concerning the adenosine triphosphoric acid content (*Fusarium oxysporum*) and muscle adenosine triphosphoric acid content (*Fusarium lini* Bolley) of these cells (p. 167 and 237, respectively). The content of organic phosphorus donors in *F. lini* was contrasted with the content of *F. graminearum* and, in turn, the data obtained with Fusarium mycelia were contrasted with the contents of yeast and *B. coli*, of the same phosphorus donors. Moreover, studies concerning the influence of added organic phosphorus donors instead of inorganic phosphorus in the qualitative and quantitative course of fermentation by Fusaria were published (p. 238 ff) and the accumulation of these donors within the cells were established, without having influenced the course of the reaction. Furthermore, the paper quoted under 65 was published in 1936 and was refuted in the same year (1) by myself without being quoted by the author. Moreover, having been published in 1936 it could not contain, in my estimation, a criticism of my data recorded in 1937 and 1939.

Due to the negligible amount of organic phosphorus donors in Fusaria, it is not surprising that the abundant pyruvic acid formation in pentose and glucose fermentations (2, 3) is not preceded by phosphoglyceric acid accumulation in the presence of sodium fluoride, when attempts to isolate this intermediary were made according to the procedure of Stone and Werkman (4). This observation is in good agreement with the results obtained and described by Semeniuk with *Chaetomium funicola*, if the latter are interpreted in an unbiased way and with the data of Huszák (5) and with the findings of Heitzmann (6), etc.—F. F. Nord, Department of Organic Chemistry, Fordham University, New York 58, New York. Communication No. 37. Received November 10, 1944.

[1] Paper by G. Semeniuk, Iowa State College Journal of Science 18, 325-358:1944.

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The Board of Editors of the Iowa State College Journal of Science dedicates this issue to Winfred Forrest Coover in recognition of his forty years of service in chemistry at Iowa State College, 1904-1944.

The board also wishes to thank the committe on arrangements, Drs. W. G. Gaessler, F. E. Brown, N. M. Naylor and J. A. Wilkinson, for their cooperation in the preparation of the material presented.



WINFRED FORREST COOVER

RECOGNITION PROGRAM

WINFRED F. COOVER, THE MAN

ANSON HAYES

Research Laboratories, The American Rolling Mill Company, Middletown, Ohio

Professor Coover was born May 29, 1875, at the Beardshear home near Vandalia, Ohio. His father, John Quincy Adams Coover, and mother, Marcella, became recognized as leaders in farming on a farm which was devoted largely to the breeding of fine horses, cattle, and sheep. Professor Coover completed the public school education and high school while living on this farm. He obtained his college education at Otterbein University, majoring in chemistry. After graduating from Otterbein, he took graduate work at Ohio State University, where he continued his specialized training in chemistry, and obtained the master of science degree.

He was employed as assistant professor in the Chemistry Department at Iowa State College in 1904. He was made professor of chemistry in 1907, and head of the department in 1913.

Soon after Coover came to Iowa State in 1904, his unusual ability, tremendous energy, and broad interest in the college activities were recognized. For many years he served on the Athletic Council and on many committees of the college. He has always been active in the Sigma Alpha Epsilon fraternity, of which he is a member.

President Friley, Dean Buchanan, and Dean Gaskill already have told you of the service which he has given in these fields. Dr. R. E. Kirk has told you of his service to chemistry. Now I find myself in somewhat of a dilemma in discussing COOVER, THE MAN, because it is difficult to talk about Coover without also talking about the services he has rendered to the college and to chemistry—all of which during my acquaintance with him have been so important a part of the Man.

The demand on chemistry in a school whose job it is to train men and women in agriculture, in engineering, in home economics, in industrial science, and in veterinary medicine is one of broad scope. Especially so when the requirements for undergraduate, graduate, and research training are considered. Vision and faith were essential in planning to meet the situation. Wisdom, courage, persistence, and patience were needed in carrying them out. Elements of endurance and an unusual degree of unselfishness also were required.

In the summer of 1914, which was the year I met Professor Coover, his office and laboratory were in the old agricultural extension building. In addition to teaching a full schedule, he was in the final struggle of getting enough of the new Chemistry Building in shape so that work could be moved into it for the fall quarter. I well remember how greatly I was impressed with the size and arrangement of the structure, for in its planning it was well abreast of facilities of the most advanced major

schools in the country. It was not completed at this time for we used ship-lap tables supported on sawhorses for most of the laboratory work. In fact, it was not completed to a major degree so far as equipment of rooms is concerned until well toward 1928, when I left the college. All this, however, fitted into a plan which took into consideration many factors existing at that time. Though many of us were impatient at times, yet we learned in due time that the plans which were being followed made for the best progress at the moment and gave time to study more soundly the detailed features of requirements over the long pull in a laboratory which was intended to serve not only the present but the future of a rapidly changing field of science.

In addition to the vision evidenced in the new Chemistry Building and other physical facilities which were provided, there was equal foresight in the plan and the selection of a faculty. Dr. J. A. Wilkinson already was on the scene with his stimulating presentation of work in analytical and physical chemistry. Dr. F. E. Brown came in 1917. During this early formative period of the department as it is now known, Drs. E. I. Fulmer, N. M. Naylor, V. E. Nelson, Ruth O'Brien and Henry Gilman were employed as members of the major faculty of the department. At this time Dr. R. M. Hixon was an undergraduate student but he had attracted the attention of members of the faculty with whom he took work and returned to the department after extensive graduate work was completed. Dr. Rachel Edgar was employed at the time Ruth O'Brien went to the Department of Agriculture, and Dr. N. A. Clark came to organize the work in soils chemistry.

These people were mostly untried but, as has now been well proved, the insight and understanding on Coover's part of the potential ability and growth of these young people have fully justified the judgment and the faith which he placed in them at that early date. The wise selection of this faculty group has resulted in Iowa State College being known for the leading authorities in the Chemistry Department in many lines of research which have been conducted here, and for the high quality of instruction given both in graduate and undergraduate subjects. Many scientific publications in the form of reference books, scientific articles, and textbooks, and the success of the undergraduate and graduate students in the field fully support these statements.

During the last ten years there has appeared a somewhat younger group including Drs. I. B. Johns, F. H. Spedding, H. C. Diehl, H. A. Wilhelm, R. E. Rundle, Sidney Fox, L. A. Underkofler, and W. B. King, who already have amply demonstrated their leadership in their respective fields.

The provision of a basic set of physical facilities and the selection of a faculty of potential growth and leadership in the many lines of work which chemistry dominates or influences, are only the beginnings of the accomplishments of the chairman of the Chemistry Department. It does, however, demonstrate the vision on his part.

There are various features, such as fostering, guiding, and encouraging this faculty, and an understanding of the individual members of it, the recognition of potentially important lines of work, and the provision for space and equipment for carrying them out—all of these also are essential. Then there is the requirement of open-mindedness in accepting good advice from the various specialists on the staff in regard to the shaping of their own lines of work and facilities.

All of these things require faith in people, wisdom in dealing with the many problems, courage to carry out the plans, patience in dealing with interfering difficulties, both in the faculty and on the outside. Frequently in addition to these qualities, plain unadulterated endurance on the part of the chairman becomes a characteristic of merit. Coover has all of these qualities to an unusual degree.

I expect that at the time I was in the department I was as much of a problem for him as any member of his staff. I know I obtained a great deal of support from him. Many times when I entered his office with some very troublesome problem, I came walking out on light air and with all the enthusiasm in the world for the solution of the problem, which was worked out during the conference.

In short, I believe that the success of the members of the staff is due not only to their own excellent abilities, but also in a very considerable measure to the provision of a setting in the department in which their talents could develop and produce the many pieces of constructive work which have been done. Coover has not only used all of his own talents but has drawn on his staff frequently for advice and counsel in their own specialized fields and on questions of broad interest to the department as a whole.

I have criticized him for taking so much of the burden of the details of operations of the department on his own shoulders. His reply to these criticisms always was that he wished to make the money that this work would otherwise cost available for the support of the department and that he wished to conserve the time of the members of his faculty to do more work in their own special fields. At times his close friends have tried to persuade him not to use his own personal funds for various features of expense around the department, always without success.

I have played golf and have gone shooting with Coover and, although I have never had the privilege of observing his behavior when I was winning, I am sure he is a most modest and gracious winner.

The features of my experience while associated with him which so impress me and which I have tried to express in the form of his vision and faith in the future of chemistry and his staff, his wisdom, courage, patience, and endurance in meeting the many problems which are encountered in accomplishing marked leadership in a field of science, perhaps may be summarized in another and possibly a more direct manner. This is the readiness and energy with which he supported the members of his faculty who, first, had demonstrated evidences of leadership; second, who

were willing to work very hard; and, third, who were engaged in projects which would develop interest of the public in the college, the department, and the faculty.

Prior to 1915, when I came with the Department of Chemistry at Iowa State, analytical chemistry was one of the major branches on which emphasis had been placed. Since that time whole industries have come into existence based on what is now known as "surface chemistry."

A good example of one of these companies is the Minnesota Mining and Manufacturing Company of St. Paul. This concern contributed to the industrial uses of chemical development, although one would not be led to expect it from the name of the company. This company is a major factor in the manufacture of the well-known "scotch" tape, an adhesive. It has developed highway warning signs which are lighted by the automobile which furnishes the light for the fluorescent effect to warn its driver. It has been an important factor in the development of flotation in its application to the beneficiation of industrial ores. In the steel business, this turns out to be of extreme importance in the beneficiation of iron ore. The supplies of the highest grades of this ore are rapidly approaching depletion, and it will be necessary to provide means of economically using much lower grades. The physical chemistry as largely developed in aqueous solutions has now been extended to a rather complete physical chemistry of steelmaking and steel processing. Free energy values are available to about as great an extent in this field as in the usual fields which chemistry had earlier served.

The application of fermentation and of chemical reactions promoted and controlled by specific bacterial action has resulted in the present great alcohol industry. Organic chemistry has contributed greatly to industrial processes of production of alcohol from crude oil and gas supplies.

All of these lines of chemical development have safeguarded the nation in the present loss of sources of supply of natural rubber.

Penicillin, which comes from controlled mold action, is now manufactured in many large specialized industrial plants, and sulfa drugs, which are the result of organic chemical synthesis, have appeared during this war period. Essentially all that is known in regard to the action of hormones has been developed since 1915, and the tremendous business of manufacturing vitamins has resulted from recent chemical development work.

The whole field of plastics which has without cause frightened managements of structural materials, although a large industry, is still in its infancy. Synthetic fuels which, no doubt, will lead to the industrial use of low grade coals, of which Iowa has so much, already are in sight. Even industrial power from nuclear decomposition can well be expected within the next half century.

The accelerative rates of industrial utilization of industrial research were well stated by Harry L. Derby, president of the American Cyanamid and Chemical Corporation, in the October 25, 1944, issue of Chemical and Engineering News. He points out that in 1920, about 300 industrial

companies in this country employed 9,300 persons in research. By 1930, these numbers had grown to 1,625 establishments with personnel of 34,200. By 1940, the figures were 2,350 establishments and personnel of over 70,000. In other words, in 20 years there was more than a sevenfold growth in the number of establishments and of personnel engaged in industrial research. Expenditures by industrial firms for this purpose in 1940 have been estimated to be about $300,000,000 a year.

I mention these things because the Chemistry Department of Iowa State has kept abreast of the demands on it during these phenomenal growths of industrial research.

A hint as to the tremendously increased demands on Iowa State College and the Chemistry Department is gained through this statement of the tremendous importance of industries which have been born within the last 25 years purely as a result of developments in chemistry. A hint of the requirements in scientifically trained personnel in chemistry comes from this same review of growth, which shows an increase in research personnel from 9,300 in 1920 to over 70,000 in 1940.

The plan of the building in 1913, the establishment of the faculty and its growth and supplementation during the succeeding years, and the recent selection of Dr. Hixon as chairman of the department, all lend support to confidence in the Chemistry Department at Iowa State continuing to be in the position of leadership which it has maintained. It has a tremendous job to do.

The final test of a man which is so rarely found is that which foresees and appreciates the necessity of having developed talent which can take over the leadership at the time when it should be relinquished in the best interests of the department. An important further step to that of selecting leadership which can carry on, is to have obtained it early enough so that it is possible to demonstrate that it can unquestionably take over and carry on with the continuing requirements of expansion and changes in facilities necessary to maintain the advanced position the department has reached. All of these things Coover has done, and it requires a degree of wisdom and unselfishness that is seldom found.

And now I wish to say that the W. F. Coover Recognition Committee is to be commended in initiating and carrying out the present ceremonies while Coover is in a position to give of his guidance and while he continues to teach the subjects in which he has had a life-long interest and experience. This he can do in the atmosphere which his life work has created. I am sure we are grateful for the kind of man he is and for the 40 years he has already served in the Department of Chemistry at Iowa State College.

COOVER, THE TEACHER

CHARLES E. FRILEY

President of the Iowa State College

In the development of her program of public higher education the State of Iowa has wisely limited the number of state-supported institutions to three. Since 1909 these institutions have been governed by a single Board. With unusual wisdom and foresight the Iowa State Board of Education has devoted its energies to the development of a unified program, assigning to each of the three schools specific lines of major educational activity. Thus, the State University of Iowa is recognized as the center for education and training in the liberal arts and the professions; the Iowa State Teachers College for the training of teachers for the elementary and secondary schools; and the Iowa State College as the center for education and training in the fields of science and technology. Within these limits the schools have advanced to high levels of standards and attainment, due primarily to the clarity of their objectives, the absence of unnecessary and unwholesome competition and duplication, and the intelligent financial support of the State.

The role of the Iowa State College in this program is distinctive. It is dedicated to the thesis that the education of young men and women in pure and applied science and in the humanities is the most effective means by which modern science can become the constructive instrument of man. It seeks to give the student a mastery of the fundamentals of the sciences in their application to the important activities and vocations of modern life; at the same time it emphasizes the necessity for an adequate background in the general studies as a basis for intelligent consideration of the shifting economic, political, social, and emotional problems of the society of which the student is a part.

I emphasize this statement of the objectives of the Iowa State College because I am convinced that the American college cannot function effectively unless its purposes are clear and its methods sound and appropriate. There is no educational problem which arouses so much discussion and dispute. The very term "education" seems to be persistently and widely misunderstood. It is too often conceived as almost entirely a process of pushing knowledge into the human mind, when in fact the word means just the opposite. To educate is not to push in, but to lead or draw out. This, of course, is elementary. The true objective is not to load the student's mind but to stimulate it. An educated man is not primarily one who merely produces facts, but one who can sort them out and think them through; and who by long training in learning, thinking, and action realizes approximately the extent of his ignorance and limitations, as well as his abilities.

It is one thing, however, to formulate a sound and acceptable state-

ment of objectives; it is another thing—equally important but more difficult—to determine what instruments should be employed, or what road should be charted, by means of which the objectives may be approached or attained. It is on this point that educators argue endlessly, usually with more warmth than illumination.

But certain fundamental principles covering objectives and means can be clearly stated and should be generally accepted. We should face frankly the fact that great expenditures have been made for imposing physical plants, costly equipment and courses of questionable educational value and doubtful educational need. We should face frankly the fact that most colleges and universities could profit greatly by periodical inventories and self-surveys, designed to eliminate archaic courses and unnecessary activities, to provide the factual bases for revision of essential activities, and to lay the ground work for sound and desirable additions to the educational program.

The fundamental agency—and by every valid measure the most important agency—in the attainment of institutional objectives is the teacher. And the greatest single need in the program of higher education is better teaching. To make teaching most effective, scholarship should be encouraged and adequate time and facilities provided for pertinent research by those members of the staff whose talents and interests clearly lie in that direction. Scholarship, research ability, and inspirational teaching are not incompatible but complementary. The Iowa State College has a large responsibility not only for research in the fundamental problems affecting its particular fields of activity, but equally for bringing students into contact with members of the faculty who are wrestling with these problems. Such a program will definitely raise the standards of the College and will give it that creative atmosphere which is tremendously important in the education of young people.

The art of teaching, which depends for its success upon quick and understanding communication between mind and mind, has suffered greatly by the amazing amount of detailed analysis to which it has been subjected during the past few decades. I do not refer to the many excellent studies dealing sympathetically with the improvement of teaching; rather, I deplore the super-analysis and the hyperdissection of the teaching process from which there has been no appreciable gain, and to which may be attributed a direct loss of some of its effective power. The so-called science of education has received such solicitous attention in the past three decades that we have grossly neglected the more important philosophy of education, which deals with values, appreciations, insights, and powers.

So much emphasis has been placed upon training and particularly upon highly specialized training that we have failed to place adequate emphasis upon the kind of man the college teacher should be. We have been so concerned with his competence in the field of his specialty that we have failed to give proper attention to his personal appearance, his manner of speech, his grammar, his cultural and community interests,

his attitude toward students, and many other things of highest importance in a teacher. Successful teaching is to a large degree a matter of personality; a man's teaching ability and power increase only as he enriches his own life, as he broadens his tastes, and as he enlarges his own horizon. In short, the first duty of the teacher is not to do something with or for the student but with himself.

It seems fair to say, then, that one of the important aspects of good teaching is the extension of the teacher's personality through the medium of subject matter. Most of us can recall, with gratitude, certain teachers whose influence, whose personality, and whose outlook on life have made an impression far more permanent than the information they imparted. I do not mean by this statement to detract in any way from the importance of what is taught. The subject matter of a course must be well organized and thoroughly authentic, and must form an adequate vehicle; it must have definite significance and merit; and it must be of such character and such pertinence that the teacher feels fully justified in dedicating all his talents and energies to its development, its interpretation, and its transmission. But the thoughtful student will not dissociate what the teacher knows from what he is.

The problem of the teacher is not new. In 1832, Alfred Tennyson, speaking of Cambridge, said:

> "Therefore, your halls, your ancient colleges,
> Your portals statued with old kings and queens
> Your gardens, myriad-volumed libraries,
> Wax-lighted chapels and rich carven screens,
> Your doctors, and your proctors and your deans,
> Shall not avail you when the day-beam sports
> New-risen o'er awakened Albion—No!
> Nor yet your solemn organ pipes that blow
> Melodious thunders thro' your vacant courts
> At noon and eve: because your manner sorts
> Not with this age, wherefrom ye stand apart;
> Because the lips of little children preach
> Against you, you that do profess to teach
> And teach us nothing, feeding not the heart."

Nearly one hundred years later President Wilkins of Oberlin portrayed the good teacher in these words:

"The good teacher knows his subject and believes profoundly in its significance, immediate or ultimate, for the enrichment of human life. He cares about his students, as thinking, feeling and growing individuals, and is glad to listen to them, in the classroom or outside the classroom. For their sakes, and because of the nature of his own mind, he selects his material rigorously and orders it effectively. His presentation has always some measure of informality, of give and take. He is courteous and helpful to all; but his chief concern is for the stimulation and guidance of his ablest students. He is a born teacher, but he is a made teacher as well—

made through friendly contacts with colleagues in his own college and elsewhere, through deliberate study of the art of teaching in his own field, and through the resolute development of all his powers."

Those who have had the good fortune through the years to study Chemistry under his direction will recognize in W. F. Coover the embodiment of all those enduring qualities, tangible and intangible, which make up the great teacher. His broad knowledge of the field, his deep sympathy for and understanding of students, their problems and relationships, his high sense of honor and integrity, his loyalty to his associates, mark him as a man whose influence will abide long and warmly in the hearts of his students.

Mr. Coover, you have been an honored member of this staff for forty years; you guided the destinies of the department for more than thirty of those years. You saw the program in Chemistry grow in size, scope, and influence to the point where it now receives world-wide recognition. Your friends here tonight realize full well that this remarkable progress is largely due to your foresight, your singleness of purpose, your courage in the face of difficulties, and your constant and high faith in the destiny of the institution to which you have devoted your life. And we, your friends, know also that through all the years you have been not only Coover, the chemist and administrator, but even more important and in the best sense of the terms, you have been and are—Coover, the man and the teacher.

We are grateful to you—more grateful than words can express—and we look forward with a deep sense of gratification to our continued close fellowship and association in the challenging years ahead.

PROFESSOR W. F. COOVER AND THE DIVISION OF SCIENCE

HAROLD V. GASKILL

Dean, Division of Science, Iowa State College

My assessment of Dr. Coover's contributions and worth to the Division of Science is to be limited to five minutes—imagine the plight of an organic chemist told to cover his field in five minutes!

No index to Dr. Coover's wisdom and foresight in the thirty-one years he has spent building a department of chemistry is better than the department's character today. He has insisted upon a well-rounded department integrated with the purposes and objectives of The Iowa State College. The staff which Dr. Coover has brought together includes distinguished men and women in many fields of chemistry. The range of courses developed under his guidance and leadership is broad, without a dissipating of strength. The only limitation he has placed, either on fields of chemistry and courses or on personnel, is that the area represented be consistent with the purposes of the College and the Division.

The curricula in Chemistry and in Chemical Technology have had all of their development—from inception to the present—under his guidance. So often course offerings and curricula stemming from a complex department can develop an inflexibility of structure and purpose, can confuse means and ends, and fail to meet new needs. From the beginning, Coover has prevented these errors. The undergraduate outline for Food Technology of some six years ago recognized new needs and evidenced flexibility. Our department's requirement of three fields of chemistry in graduate programs manifests the insistence upon integration. His demands for quality of performance for both undergraduate and graduate are well known to us all. Degrees in Chemistry since 1913 are, roughly, B.S. 330, M.S. 175, Ph.D. 260.

He has been uniquely successful in providing in his department an environment designed to enhance the spirit of enthusiastic teachers and ardent research workers. As a department head he has mastered the ability to develop initiative and a feeling of responsibility on the part of men and women of his staff.

A list of his other general services to the Division is long indeed. Many years of valuable aid were given our Curriculum Committee, the Athletic Council, the Division's Science Day, Divisional Personnel Committee, and many, many others.

Parents, employers, and society at large demand a great deal more of chemistry graduates than education in chemistry and intellectual training. They must be fine people and worthy citizens. Teaching the value of consummate honesty and indomitable character by precept has been

Professor W. F. Coover's forte to a degree which, in my opinion, has not been excelled anywhere.

I count myself fortunate for having been associated with him as Head of the Department of Chemistry and look forward to his continued service as Professor of Chemistry.

THE ORGANIZATION OF A COLLEGE CHEMISTRY DEPARTMENT

RAYMOND E. KIRK

Polytechnic Institute of Brooklyn

Mrs. Kirk and I are indeed very happy to return to Iowa State College to share in this occasion. We pay tribute to the college and to Professor Coover for their influence on our lives and our careers. I came to the campus in 1915 as a graduate student and returned to it after the last war to serve briefly as an instructor. Those were formative years; and the ideas gained then have been potent in giving direction to the following years.

It is a pleasure to speak on the topic assigned to me both because it is one in which I have a vigorous interest and because it is one that has been so well exemplified in the distinguished career of our honored guest. I shall talk in general terms. He has thought in general terms and then he has reduced these ideas to practice. The success of this endeavor is attested to by our meeting tonight, a meeting to celebrate the continuing success of a strong department of chemistry.

The first general principle in the organization of a college chemistry department is that the department of chemistry must stand on its own feet. It must have an identity of its own. It must have a characteristic flavor and a distinctive personality. In order that a college chemistry department may perform its function as a servant, it must first of all be a master in developing its own point of view, its own pattern of thinking, and its own contribution to science.

Reflecting upon the place of chemistry in the land grant college system, one realizes that to a very large extent the land grant colleges were built around chemistry and around chemists. Read the names of the early directors of agricultural experiment stations! Note the names of the early presidents of the land grant colleges! Leaf through the faded pages of the old catalogues! The names of chemists are at once to be seen in the majority! Chemistry was the key subject! There was indeed, a reason for this. The science of chemistry was a well organized body of knowledge. It had in its ranks many competent and well trained persons available to apply the experimental method to the problems of agriculture. The traditions of Liebig and of the famous Rothamsted Station were well known to chemists of that day. The profession of chemistry had enlisted many of the most vigorous minds of the time. Many of them had obtained European training and had then returned to the United States to use that training. They found little outlet in industrial fields but ample opportunity in the rapidly developing experiment stations of the land grant colleges. Each of the early experiment stations had a coterie of famous chemists. Each land grant college was tremendously influenced by its vigorous chemists and by their enthusiasm for the profession of chemistry.

Then there came a time when chemists and chemistry became somewhat submerged in the work of the land grant college. Other and equally important disciplines had been developed. Vigorous and enthusiastic investigations were going forward in borderline fields between chemistry and the various biological sciences. The emphasis in biological science was shifting from form to function; from morphology to physiology. Along with this, unfortunately, there had developed some serious defects in the work of chemistry and of chemists in the land grant colleges. There had come to be an undue formalization in the chemical instruction. The courses in chemistry offered to students placed emphasis upon descriptive chemistry rather than upon fundamental principles, thus repelling vigorous young minds. Chemistry was "just analysis". "Dry rot" was making its appearance in chemistry departments and in chemical sections. Inbreeding was prevalent largely because of salary schedules.

Then too the land grant colleges were faced with the necessity for the training of a large number of extension workers, of demonstration workers, and of vocational teachers for the high schools. The laboratory scientists, especially in chemistry, became submerged in all of this. Departments of chemistry became merely service departments for the training of such workers without simultaneously pushing vigorous investigational problems of their own. Worst of all, the chemical industry was, by that time, drawing off a great many of the young men coming out of graduate schools of chemistry. This occasioned the shift of many of the ablest minds in chemistry away from academic institutions, especially those not closely affiliated with vigorous research in the fundamentals of chemistry. Physical chemistry and modern organic chemistry were not represented in the programs or on the staffs of many land grant colleges. This was the era of "hyphenated chemistry."

Now we are coming to a period closely related to my topic and to the career of our honored guest. This is the period of the rebirth of departments of chemistry in the land grant college. Dr. S. W. Johnson, of the famous Connecticut Experiment Station, once put it this way, "If one wishes the respect of others, he must first earn his own respect." This leads us back to our first general principle. The college chemistry department cannot occupy its proper place in an institution unless it has an entity of its own. It must not only train men who are to use the knowledge developed in chemical laboratories; it must also train men to develop knowledge through chemical investigation.

The college chemistry department cannot have on its staff good teachers unless it does major work in chemistry at all educational levels. It cannot earn the respect of its sister departments in the college unless it first of all earns its own respect. This is the fundamental premise on which the college chemistry department must be organized.

The second general principle in the organization of a college chemistry department is that the whole is greater than the sum of its parts. We are not discussing geometry. We are discussing something far more complex. Our experience justifies noneuclidean statements. Each general subject

matter division of chemistry will, in the well planned college chemistry department, be organized vertically from undergraduate years, on through to graduate years, including research at all possible levels. This will be done, and must be done despite the usual and desirable horizontal organization of student registration, student direction, student guidance, and student regulations. It will be done and must be done despite the conflicting details of budgets and research assignments. It must be done this way if chemists are to be well trained and chemistry is to be well served.

Along with the vertical organization of subject matter the training of students and the organization of research must be completely integrated. This is important for the best interests of minor students and of major students. The chemist is first and foremost an investigator! He must be! Investigational problems demand the use of all subject matter developments and of any and of all possible experimental techniques. There are no subject matter divisions in research.

The fundamental principle of vertical organization of subject matter with horizontal integration of the science extends to all of the applications of chemistry to the far flung interests of the investigational programs of the land grant college. If an investigator in a related field needs help from chemistry and from the chemist he should first of all decide where he wishes help, at the *technician* level, or at the *scientific* level. No chemist can give his best to an investigational problem if he is to be considered only as a machine for analysis. Routine tests should be made by technicians, not by scientists. *Scientists* are too precious a commodity to be wasted in this fashion. When routine tests are needed they should be made under the direction of a responsible investigator by a technician. When *scientific* help in chemistry is needed it should come from a well organized, thoroughly integrated department of chemistry. The liaison chemist can then marshall all the resources, methods, and disciplines of the science back of the project. A college chemistry department of this sort will very soon establish that the whole is greater than the sum of its parts.

Such a department will contribute to the educational program of the college. It will contribute to the research program of the college. It will add prestige to the college and to the state.

The third principle in the organization of a college chemistry department is that a scientific organization consists of men. It is not created by a neat outline of administrative responsibilities carefully framed and placed in an executive office. Organization means men. Direction of that organization involves vigorous leadership. I scarcely need elaborate on this aspect of the college chemistry department to this audience. You have seen it work! Able men must be chosen. They must be selected from diverse backgrounds and with very different preparations. They must be brought together, indoctrinated and encouraged to work out together the real problem of organization, which is "what each man can do."

One of my former students employed at the time in a large research laboratory operated by one of the major chemical companies was once

asked by an administrative superior, "Are you the section leader?" My friend and former student replied, "I do not know, am I?" The question came back, "Don't the men in certain groups always talk to you about their work?" "Yes, they are my friends." The pay-off is that this young man had been carried on the budget as a section leader for two years before he found it out. He had not bothered to find out, he had just gone ahead with his chemistry. He now holds a very high position of administrative responsibility with the company. The way to be a leader is to be one. The way to do chemical work opens up before the worker.

It is easy to write the prescription for the college chemistry department that good young men should be obtained for its staff. That is not enough. They must be kept enthusiastic both about their teaching and about investigation. Their needs must be supplied with respect to equipment, with respect to supplies, perhaps even with respect to stenographic assistance. Graduate students must be turned in their direction. Special research assistance must be obtained, vital as it often is to the development of special interests and abilities. Finally the men must be *pushed*. I need not elaborate on this topic before this audience. Too many of us have keen memories of how the push got clear down to the level of the graduate student.

The fourth general principle is that the work of the department must be integrated with the needs and interests of the state. The best crop in Iowa has always been the young men and women who crowd the laboratories, libraries and classrooms of its colleges. Their vigor must be given adequate outlet, their enthusiasm must be given proper direction. The Department of Chemistry at Iowa State College has long stood for "science with practice." In it thousands of men and women have trained for vigorous leadership in the profession of chemistry. Additional thousands have been given guidance in the use of chemistry in related professions. All of these men and women meet with us in spirit tonight in tribute to Professor Coover.

The scientific work of the college department of chemistry must also be integrated with the industrial and agricultural possibilities of the state. Need I review here the achievements of the department in such endeavors?

We meet here tonight to review the past and to predict the future. But the permanent record of the achievements of our honored guest is written on the minds and hearts of thousands of his former students now scattered round the world. Eyes front! Heads up! Coats off for the future of science!

FORTY YEARS OF PROGRESS

INTRODUCTION

JULIAN HARRISON TOULOUSE

Chief Engineer, Quality and Specifications Division, General Manufacturing Department, Owens-Illinois Glass Co., Toledo, Ohio

Perhaps we are opening our meeting a little formally, but this is no ordinary meeting. To plan to honor Professor Coover by a scientific program is very nice; to be able to honor him by bringing together five of his students who have made names for themselves in so many diverse fields, is something for all of us to be proud of.

One of these students is a leader in the field of chemical engineering, particularly in high-pressure metallurgy. Another leads in food chemistry, particularly in the development of baby-foods. A third is engaged in that intensive research drive in synthetic rubber. Another has gained national recognition in the control of insect-borne diseases and infestations, particularly in the war-time use of the fumigation bomb. The fifth is a leader in the farm chemurgic move, a program of interest both to the farmer and the chemist.

We speak of these people as Professor Coover's students, but I am not sure that any of them actually took class work under him. They are his students in a much broader sense; it was his organizational work that made their studies here both possible and profitable. That is the phase that I want to touch upon before opening the real part of the program.

The organizational aspect of teaching is not usually given the credit it should receive. We take too much for granted; we begin to think that all you have to do is to give a teacher a classroom and some students and the rest comes as a matter of course. Since I happen to be employed in an establishment which because of its large size is highly integrated, I have learned at first hand the value of this thing called organization.

If we are skeptical about the importance of organizational work, we can learn something by comparison with the present emergency. Generals Eisenhower, Somervell and Marshall are said to be the leaders they are, not because of any particular ability in combat, but because of their ability to organize.

There is no real secret about the leadership in chemistry teaching this department has taken. When, for an altogether too brief a time, I served Professor Coover as an assistant in his office, he told me one of the reasons why the Chemistry Department of Iowa State College is so outstanding in teaching. That reason is: he has never allowed the department to become inbred, or to follow too closely any one school of thought.

That means that his teaching staff was developed, and enlarged as time demanded, with the utmost care. Every important school of chemistry has been represented, and with outstanding people. No one-sidedness has been allowed to develop. This has not meant the exclusion of our own

outstanding students—many are to be found on the staff, but balance has been carefully maintained. That such efforts have borne fruit is shown by the leadership his students have reached in their respective fields.

Another monument to Professor Coover's organization is this building. As chemical laboratories go, it is getting along in years. It was one of the first of its kind, designed for extreme flexibility in arrangement and alterations. It is possible to make a large number of changes simply by removing curtain walls. It was built for the future and has kept pace with the expanding demands. No doubt improvements could be made now, which were not known some thirty years ago. Perhaps some of the facilities are being outgrown, but the planning job done by Professor Coover over thirty years ago, and the efficiency of the building that was the object of his planning were the results of real organizational ability. The arrangement of the main lecture room, its availability to the principal entrance, the organization of the laboratories in relation to the store rooms, all are on a plan I have not seen equalled. It is a perfect example of how mass-teaching and research can be carried on together without mutual interference.

Because of this organization, the Chemistry Department has been of utmost assistance to all the divisions of the college that needed its centralized service. In other words, it was not only designed to serve chemists, but chemistry. Without it our agricultural students, our engineering students, our home economists, and our science students might have been studying chemistry in isolated groups which, even though they might have been efficient and well led, would not have had the strength, or the facilities, or the technical completeness of this single unit.

Yet each of these groups has had its own thoroughly organized staff. None was thrown into a melting pot (or should I have said crucible) with a mixture of other divisions and other divisions' needs, until the finished product in students was an alloy of no particular specialization, yet released to a specialized field. I can speak of the thoroughness of this in mentioning that my wife and I, (we entered together as freshmen two years after our marriage), one taking work in agricultural chemistry, the other in chemical engineering, did not once cross paths in relation to either courses or teachers. Our one hardship was in not being able to caution each other on the foibles of our instructors.

I have taken advantage of the position of presiding officer to pay this personal tribute to Professor Coover. It is in the nature of paying a debt to his teachings and counsels. Since all of us on this program use the letters D and R in our titles, might it be equally applicable today as the initials of the word "debtor"?

With this thought in mind, it is now my privilege to introduce the first paper of this program of scientific papers by students of Professor Coover.

TWENTY-FIVE YEARS OF RESEARCH ON FERMENTATION PROCESSES IN PROFESSOR COOVER'S DEPARTMENT

LEO M. CHRISTENSEN

University of Nebraska, Lincoln, Nebraska

INTRODUCTION

Those of us who have had parts in this research program on fermentation processes have a feeling it has occupied a special place in Professor Coover's plans, and have justified that opinion on the basis that fermentation processes are so closely related to agriculture and so important to it. All of us know that in building the Chemistry Department, Professor Coover aimed at service to Iowa and the Midwest above all else, and he built his staff around that ideal. Graduates who have had the privilege of doing research on fermentation processes in this department have known of the care with which Professor Coover selected the man to direct this program, and have seen his good judgment fully confirmed. Today I am honored with this opportunity to tell you about the research that Dr. E. I. Fulmer has directed as his part of the program of service that Professor Coover set up here more than twenty-five years ago.

At the outset the term "fermentation process" should be defined, but that is not easy to do. The expression developed by Dr. Fulmer, "microbiological dissimilation products of the carbohydrates" is, to say the least, intriguing. But in the end we have usually considered a fermentation process one in which chemicals are produced from farm crops by the action of yeasts, molds, and bacteria, and let the matter rest there.

From the research standpoint it is pertinent to use another of Dr. Fulmer's characterizations, "autocatalysis in a heterogeneous system," because it so aptly describes a general basic problem which the zymologist must constantly keep in mind. In studying the influence of any one factor upon the operation of a fermentation process, he is concerned with the influence upon the catalyst formation as well as its action. It is a rare case when one can be entirely separated from the other. Add to that the difficulty that the chemical nature of the materials entering the process is still not well established and it can be appreciated that the operation of a fermentation process on laboratory or plant scale is both an art and a science.

EARLY RESEARCH WAS CONCERNED WITH DEVELOPMENT OF THE CATALYST

Man has used yeasts almost since he quit living in a cave, to make his wine, brew his beer, and to raise his bread. One of the classic debates of science was that of Liebig and Pasteur concerning the function of yeasts in these old processes. But in spite of the long association with yeasts, man's knowledge of them is still quite meager.

A new approach to a better knowledge of yeasts was early developed at Ames. It was based upon a simple theorem—if you want to know something about a biological form, ask it. The questions asked must be of the type that get a yes or no answer, and must be carefully planned. First, a certain strain of *Saccharomyces cerevisiae* was selected as the test organism, and it was cultured in a standard manner. Then it was put into media in which one single component was varied, in a series selected to extend above and below an optimum. Yeast population was the measure used in these studies. A systematic application of this method developed a great deal of new information about yeasts, and some principles that seem to be widely applicable to biological systems.

Thus it was found that the ammonium ion is only a little less active than are hydrogen and hydroxyl ions in their effect upon yeast growth. Furthermore, for each temperature there are two definite optimum ammonium ion concentrations, not affected by other components of the substrate. Both are linear functions of the temperature and this relation is described by the following equations, where t° is in °C.

(1) Optimum normality $= 0.00179 + 0.00057\ t°$
(2) Optimum normality $= 0.111\ \ + 0.0042\ \ t°$

These formulae were found to be more fundamental in character than was at first anticipated. Thus in the production of citric acid by *Aspergillus niger* in a synthetic medium, at 30°C., the optimum ammonium ion concentration is exactly that forecast by the formula derived from the studies on yeast. And in the thermophilic fermentation of cellulose to yield ethanol, at 55°C., the actual optimum and that forecast by the formula are the same. Furthermore, it was found that over a wide range of temperature, both in synthetic media and in beer wort, the ammonium ion concentration optima calculated for yeast growth are the same as those at which wheat gluten is least hydrated.

A study was made of the influence upon yeast population of many so-called growth stimulants, and the multiple nature of bios was clearly established long before this was reported from other laboratories. Another fundamental principle in biological studies was uncovered in this work. It was very clearly demonstrated that when substances are compared as sources of growth stimulants, they must be compared when each is used at its optimum concentration, and at an optimum temperature, not at arbitrarily selected equal concentrations and temperatures.

During these studies it was noted that yeast would grow in synthetic media containing so little of nitrogen sources that there was a question whether any but elemental nitrogen was available. Still the yeast contained the usual amount of protein. An intensive research program showed that under certain conditions yeast is able to build its protein from atmospheric nitrogen, in amounts and at rates like those found for Azotobacter species.

The importance of available phosphorus received its proper attention. Up to an amount required to supply the phosphorus needed to form cell

protoplasm, there is a linear relation between phosphorus content of the medium and yeast population, but beyond that point the phosphorus content of the medium is without effect, other conditions being equal. It was a source of astonishment how efficient yeast is in transferring phosphorus from the substrate into its own cell substance. Even with highly purified ingredients, with no added phosphorus, yeast is able to grow, getting its small phosphorus requirements from a substrate containing only an infinitesimal amount of it.

The influence of calcium, magnesium, sodium, potassium, iron, and other ions, antagonistic actions among these ions, and other factors in yeast growth were systematically studied, and fundamentally important information developed. Here is a method that deserves much more attention among biologists than it has received. Systematic questioning of the biological form, using this prosecuting attorney technique, can reveal much that is only a mystery today.

DEVELOPMENT OF THE AGRICULTURAL DEPRESSION SHIFTED THE EMPHASIS

A service institution is sensitive to the needs of its constituency, and with the development of the serious agricultural depression in the early 1930's, the fermentation research program was tailored to the needs of the day. Making fermentation chemicals from farm crops was the new keynote, but the systematic question and answer technique still prevailed.

Investigation revealed that industrial alcohol manufacture was one possible constructive use for the great national starch surplus. True, grains had not been able, in normal times, to compete with blackstrap molasses as raw material for this industry, but might not this situation be changed by research? Although almost no modern research had been done in this old chemical industry, it seemed likely there might be something of interest.

An excellent opportunity to evaluate the orthodox processes for making grain alcohol was furnished by the investigation set up by the late Francis P. Garvan, of the Chemical Foundation, in 1936. At Iowa State and elsewhere, research had been directed toward an exact evaluation of alcohol as an automotive engine fuel, and as a result some rather pointed arguments had developed concerning the cost of making alcohol from grains. Mr. Garvan said he was tired of listening to such idle debate and set out to get a definite answer. At the same time, he said, it could be ascertained whether there were opportunities to improve the economy of making alcohol from the starch of grains.

One man from the University of Idaho and five from Professor Coover's fermentation research program were assigned the job of getting this information for Mr. Garvan. A closed small distillery at Atchison, Kansas, was borrowed and was remodeled and enlarged, to make it an orthodox but modern grain alcohol plant. For two years various orthodox procedures were applied, to get Mr. Garvan the data he had requested. The following conclusions were then announced:

1. Whereas statements from various sources had placed the cost of alcohol from corn costing 50 cents per bushel at from 10 cents to $1.00 per gallon, the actual cost was 25 cents per gallon, using strictly orthodox processing and including all reasonable capital charges.
2. Whereas it had been said that there were no opportunities for research to improve the economy of making industrial alcohol from starchy substrates, actually there were two very large processing costs, and many small ones, that research could certainly eliminate.

These two major inefficiencies were described in several reports, both technical and general, as follows:

1. For every 90 pounds of grain, 10 pounds of malt were required, malt costing 2 cents per pound more than grain and therefore contributing 4 cents per gallon to the cost of the alcohol.
2. The alcohol, carbon dioxide and total residual solids obtained failed by 15 to 20 per cent to account for the grain put into process, a loss equal to around 6 cents per gallon of alcohol processed.

The first problem was intensively investigated in Dr. Fulmer's laboratory, and a series of reports by him and by Dr. Underkofler tell the story of the development of simple and inexpensive methods for the manufacture, at the alcohol plant and under exact control, of fungal amylases that almost completely eliminate the cost of saccharifying starch, and at the same time improve alcohol yield and reduce contamination hazards. If mold bran were used in place of malt in the present grain alcohol program, it would save the government $20,000,000 per year.

The second problem was undertaken in a cooperating research project at the University of Idaho, where it was ascertained that the loss was in the carbohydrate fraction of the raw material, resulting from a hitherto unsuspected chemical change in cooling starch gels. Several means for avoiding this undesirable starch reaction were developed and as a result the products were stoichiometrically equivalent to the reactants. If these improved mashing procedures were used in the present program, they would save the government $30,000,000 per year.

Between 1932 and 1939, Professor Coover's fermentation research group tried to tell the public that there would shortly be a vast increase in the need for industrial alcohol, one that could not be supplied from blackstrap molasses and other accustomed sources. Apathy, skepticism and opposition were the answer. Beginning in 1940, that national need has arisen. Whereas in 1940 only about 5 million gallons of industrial alcohol were made from grain starches, the 1944 production from this raw material will reach 500 million gallons, an increase of 100 fold in four years. And so acute is the shortage that all industrial alcohol produced is taken by the government.

But still apathy, skepticism and opposition have prevented full commercial application of the research results described above. In one Midwestern plant, however, there is an unprejudiced and enterprising management that is, as rapidly as possible, putting this research to work, with the help of several of the technical pioneers. It is only a matter of weeks now until commercial demonstration of the research accomplishments will be at hand. In the meantime, you may be sure, this plant management has fully satisfied itself of the utility of these research accomplishments.

Professor Coover, we think you will be pleased with the results of this research project. We believe it is the kind of job you have wanted your department to do.

AGRICULTURAL WASTES MAY FIND USE IN FERMENTATION INDUSTRY

It has long been known that from such agricultural wastes as corn cobs, plant stalks, grain hulls and bran, mixtures of pentose and hexose sugars can be obtained in yields of from 20 to 40 per cent by weight using a simple and inexpensive hydrolysis with dilute acids. Quite naturally the fermentation research workers have been interested in these raw materials.

Methods for improving the ability of *Cl. acetobutylicum* to utilize the sugars of these hydrolysates were developed, and good yields of n-butanol, acetone and ethanol were obtained. It is interesting to note that this organism produces from xylose the same products it makes from hexoses, in the same ratio and with the same yield in per cent by weight.

An intensive study was made to determine the best conditions of acid concentration and time and temperature of digestion for the production of these pentose and hexose sugars from several agricultural wastes.

Research in cooperating laboratories is extending this work. Thus in one it has been found that when certain oilseed crop residues are properly digested, the residue is highly satisfactory as a source of paper, alpha cellulose and plastics, and the combined value of the sugars and the residue may develop a commercial utilization of this research.

2,3-BUTYLENE GLYCOL AN INTERESTING FERMENTATION CHEMICAL

It has long been known that many bacterial species produce some 2,3-butylene glycol from a wide variety of carbohydrates. A systematic investigation in Dr. Fulmer's laboratory showed that when the carbohydrate concentration and other factors were correctly adjusted, the yield reached 50 per cent by weight of the carbohydrate consumed, and this aroused new interest in the process.

Research on the production of the glycol and its conversion to butadiene has recently been very active, both here and in other laboratories. Since about 1912, 2,3-butylene glycol has been recognized as a good source of butadiene for synthetic rubber. But the organic chemist sees many other good uses for this four-carbon compound with two hydroxyls so conveniently placed.

MICROBIOLOGICAL OXIDATIONS ARE DELICATELY BALANCED

When the chemist looks over the chemical results of microbiological activities, he is impressed with the fact that these living forms, in attaining their life objectives, have a remarkable facility for gently moving oxygen atoms from here to there.

Aerobacter suboxydans was found to be a convenient tool to study some of these microbiological oxidations. This research has been especially active under the direction of Drs. Fulmer and Underkofler during the past five years. With approximately quantitative yields, sorbose is converted to sorbitol, mannitol to levulose, glycerol to dihydroxyacetone, and 2,3-butylene glycol to acetylmethyl carbinol. From *meso*-erythritol, 90 per cent yields of 1-erythrulose are obtained. From *i*-inositol, which has six secondary alcohol groups, a diketo-*i*-inositol is produced.

This *Aerobacter* exhibits a high degree of specificity in its oxidation of 2,3-butylene glycol. The fermentation product using *Aerobacter aerogenes* is slightly dextro-rotary, and contains about 90 per cent of *meso*-glycol. *Aerobacter suboxydans* converts the *meso*-glycol to acetylmethyl carbinol, leaving the dextro-glycol behind.

There is certainly no need for me to point out here how useful a tool has been forged.

FERMENTATION INDUSTRIES SHOULD BE AN IMPORTANT FEATURE OF POSTWAR ECONOMY

American agriculture has increased its productivity 23 per cent since the war started, using nearly 10 per cent less land and working under serious handicaps of machinery and manpower shortages. At a rate never before witnessed, the splendid research accomplishments of the Agricultural Experiment Stations are being put to use. Better crops, better land use practices, and many other developments from agricultural research are paying big dividends in this war period.

For comparison, look at the agricultural program of World War I. A 5 per cent increase in productivity was obtained, almost wholly the result of plowing new land, land that since has had to be returned to grass.

What about postwar markets for this increasingly productive and efficient farm factory? Fermentation industries can help a great deal in providing a market at a profitable level. The industrial alcohol expansion of this war period has amply demonstrated that fact. And please bear in mind that these fermentation processes use only the starches and sugars of the farm crop, products of photosynthesis; all the nitrogen and other elements of soil fertility are recoverable and can be returned to the land from which they came.

And so, Professor Coover, this research program on fermentation processes which you established here at Iowa State more than twenty-five years ago is not only doing its share in the war effort, but it should be even more important in the years to come, helping to make profitable use of the production of an ever more efficient agriculture, creating opportunities for new productive employment in industry, and establishing a higher standard of living for all.

NEW DEVELOPMENTS IN INSECTICIDES

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The tremendous scope of this war has greatly increased the insect problems of our armed forces over those heretofore encountered. This fact was recognized very early, and steps were taken to provide adequate facilities for the Bureau of Entomology and Plant Quarantine to develop and test new insecticides. A laboratory for this purpose was first set up at Orlando, Florida, with funds from the Office of Scientific Research and Development; later this program was extended to other laboratories in this bureau and to some universities. Preventing the spread of malaria, yellow fever, dengue, and filariasis by the mosquito, of typhus by the louse, and of dysentery by the fly were serious problems that were given to the chemists and entomologists for solution.

Before the war research on insecticides proceeded slowly. There was no acute need to spur this study in peacetime. When the war broke out, the fly spray used twenty-five years ago was almost the only weapon available for fighting adult mosquitoes and houseflies. No good louse control was known, and repellents had not attracted much attention. The war has not only given an impetus to the development of better insecticides, but has made it necessary to find substitutes for those made scarce by the cutting off of supplies from abroad. Some of the accomplishments in this field have recently been pointed out by Roark (24).

The recent developments in insect control include DDT, various repellents, improved louse powders, improved mosquito larvicides, organic thiocyanates, synergists for insecticides, new methods of making pyrethrum extracts, new fumigants, and the aerosol method of applying insecticides.

DDT

The most outstanding new insecticide to come into use during the present war period is the synthetic compound 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane. For convenience it is called DDT, from the initials of the generic name, dichloro-diphenyl-trichloroethane. It was first prepared by Zeidler (33) in 1874 by condensing anhydrous chloral and chlorobenzene in the presence of sulfuric acid. It is a colorless, almost odorless, crystalline solid, melting at 109°C. It is insoluble in water but soluble in most organic solvents. Cyclohexanone, the best solvent, will dissolve more than its own weight of DDT.

DDT was found to be a good insectcide about five years ago by Müller (23) in Switzerland, who reported its effect on flies, moths, and aphids. Its contact and stomach-poison action and the lasting effect of the residue

on flies were reported in Switzerland (32). Its toxicity to the body louse on man was also noted there (8); a formula containing it was later developed in this country for use as an Army louse powder (2). The insecticidal use of DDT is now covered by a number of patents in Switzerland, England, and the United States.

DDT is approximately as toxic to flies as the pyrethrins, but it has a very slow paralytic effect. Where a rapid paralytic action is desirable, either a thiocyanate or pyrethrum must be added. It is much less toxic to adult mosquitoes than the pyrethrins.

DDT is effective against a wider variety of insects than any other synthetic organic insecticide so far tested. It fills several gaps where there has been no effective control. For example, it can be considered the almost perfect answer to the bedbug menace (20), and it is an excellent chemical control for the Japanese beetle. Against many insects it promises to give better control than the insecticides previously recommended. One part in 100 million parts of water or 0.1 pound in oil per acre of surface is stated by Stage (29) to give excellent control of mosquito larvae. The effect of DDT on many different insects was reported in the February, 1944, issue of the Journal of Economic Entomology.

The excellence of DDT has naturally aroused interest in its chemical isomers and analogs. The compounds containing methyl, methoxyl, bromine, and other substituents in the place of chlorine have been made and tested for toxicity to insects, but preliminary tests show that DDT is the most toxic member of the group so far prepared. Little is known of the isomers of DDT formed by changes in the position of the chlorine on the benzene rings.

Among other synthetic insecticides, phenothiazine (28) xanthone (27), phthalonitrile (25), and certain thiocyanates have given control of some insects, but in most cases they have been much less effective than DDT. Phenothiazine has proved to be a very effective anthelmintic (16).

REPELLENTS

The repellent method of combating insects is very old. Probably smokes were the first repellents. Later certain essential oils, such as citronella, were commonly used, but not much organized research was done to find better repellents. Shortly before the war interest in repellents was beginning to pick up, and several proprietary preparations appeared on the market. In 1937 Kilgore described a method of measuring the repellent action of chemical compounds on houseflies (18) and he was granted a patent for Indalone (alpha, alpha-dimethyl-alpha'-carbobutoxy-gamma-dehydropyrone) (17). At the outbreak of hostilities great emphasis was placed on this method of protection as a means of preventing the spread of malaria. Research was undertaken by the Bureau to prepare chemicals and test their repellent effect on insects. Those showing promise were then tested for toxicity to warm-blooded animals. One proprietary product already on the market, a mixture of butyl carbitol and its acetate, was eliminated because of its toxicity to man by absorption

through the skin, but a substitute was soon found in 2-ethyl-1,3-hexanediol, known as Rutgers 612 (21). This compound, Indalone, and dimethyl phthalate, patented by Moore (22), are three good repellents. A mixture of these, patented by Travis and Jones (31), will give protection against bites for several hours.

No repellent has yet been found that gives perfect protection. There is considerable specificity, and what repels one species of insect may not repel another. Moreover, a chemical effective on one man may fail utterly on another. The conditions under which they are used also influence the repellent action and the time of protection.

SYNERGISTS FOR INSECTICIDES

One of the most fascinating subjects included in the studies on insect poisons is synergism. A synergist is a material which, when present with an insecticide, gives a mixture whose toxic effect is greater than the sum of the effects of the two materials acting independently. It may or it may not have any toxic action of its own. Since pyrethrum is relatively expensive, most of the synergists have been developed for use with it. The first commercial synergist was N-isobutylundecyleneamide, IN-930 (24). This compound increases the action of pyrethrum on flies considerably, and on lice attacking man the increase is more than 50 times. This combination was used in the Army louse powder (3) before DDT was introduced. Among other substituted amides (9) that have been tested, benzamide and piperonylamide show considerable synergistic action with pyrethrum in fly sprays.

Sesame oil (5), containing sesamin (14), is one of the best synergists for pyrethrum. It has found extensive use in the insecticidal aerosols.

The action of synergists is usually highly specific. A chemical that is a synergist for pyrethrum is not necessarily a synergist for rotenone or other insecticides. Also, the synergistic action varies greatly with the insect species and may be entirely absent with some. The search for synergists must be made for the most part by the trial-and-error method, since very little is known about their physiological action.

BETTER PYRETHRUM EXTRACTS

For use in sprays no attempt was made to refine pyrethrum extracts, because most of the inert materials extracted along with the pyrethrins were soluble in the oil used for extraction. With the advent of aerosols containing Freon-12, which will be described later, refined pyrethrum extracts became necessary in order to insure better solubility in Freon. All the companies extracting pyrethrum have greatly reduced the amount of materials insoluble in Freon-12. Specifications now require this amount to be less than 4 per cent. They have also improved the color and removed most of the substances irritating to the nose and throat.

Barthel and Haller (1) have perfected a new process of making mixtures of pure pyrethrins. They have found that nitromethane, which is immiscible with kerosene, will extract practically nothing but pyrethrins

from a kerosene extract of pyrethrum flowers in a two-phase liquid extraction. Upon further clarification with activated carbon 100 per cent total pyrethrins can be obtained.

FUMIGANTS

Of the fumigants, methyl bromide has had the greatest expansion in use in recent years. It is now employed to fumigate many kinds of vegetables, especially in refrigerator cars, against Japanese beetles and other insects. It is also coming into use on grain and mill products because of its unequaled penetrating ability.

The war has brought methyl bromide into use as a fumigant for delousing clothing (19). It is more readily applied, quicker in action, and less injurious to clothing than the steam-sterilization process used in World War I. This fumigant is also 100 per cent effective against bedbugs and their eggs, even acting through a woolen blanket. It is applied to clothing in bags or other closed spaces by breaking a glass ampule containing 20 ml. of the liquefied gas, a technique developed by Latta (19). Millions of these ampules have been filled for the armed forces.

A new soil fumigant known as DD mixture has aroused much interest lately. It consists primarily of 1,2-dichloropropane and 1,3-dichloropropylene obtained as a byproduct in the manufacture of allyl chloride. It has been used most successfully in Hawaii for killing nematodes in the soil (4) where pineapples are grown. It has given promise in California against wireworms (30). Both these pests are destructive enemies of growing crops, and a good material for their control will be of great value.

INSECTICIDAL AEROSOLS

Even before the war, work was in progress at the Beltsville Research Center to find better methods of applying the spray type of insecticide. Goodhue and Sullivan (10, 13) suggested eliminating the liquid carrier and applying the insecticide in the form of a smoke, or aerosol. In this form the insecticide stays suspended and active much longer than when applied as a spray. Tests have shown (6) the aerosol to be more effective after 20 minutes than a spray is after 5 minutes.

There are numerous ways of producing aerosols, but the most convenient is the one employing a liquefied gas (10). It is only necessary to dissolve the insecticide in the liquid under pressure and release it through a small orifice into the atmosphere. The aerosol is produced by the rapid boiling of the liquefied gas, which merely furnishes the energy for the dispersion of the insecticide and plays no further part.

Many insecticides and liquefied gases have been used in this method, but the most popular combination has been pyrethrum and sesame oil dissolved in dichlorodifluoromethane, a refrigerant known to the trade as Freon-12. This aerosol is now extensively used by our armed forces for the control of mosquitoes. It is well adapted for use on airplanes, since it is nontoxic to man, noninflammable, light, convenient to use, and very effective. Only 5 mg. of pyrethrins per 1,000 cubic feet in aerosol form is

required to kill all yellow fever mosquitoes with an exposure of only 1 minute.

The experimental work required to develop the aerosol method was easily done with commercially available equipment. Before the Army could use it, however, small, light containers had to be devised. A large electric company using Freon in household refrigerators developed the first practical dispenser. It consists of a sealed container holding 1 pound of the solution, which is dispensed through a capillary running from the bottom on the inside and out the top. To open the container the protruding capillary is broken off while the container is held in an inverted position. A temporary cap is provided to retain the contents between sprayings. This company has made and shipped several million of these 1-pound dispensers. Other companies entered the field later and have produced large quantities of the aerosol solution in various types of containers.

DDT in combination with pyrethrum in the aerosol has been found to be very effective, especially on flies (11). With this combination it is possible to produce aerosols equally toxic to flies and mosquitoes. With some of these solutions only 3 seconds' spraying (2.5 grams of solution) will produce enough aerosol to kill all the flies, mosquitoes, and many other species of insects in 1,000 cubic feet. Considerably more is required for crawling insects, such as roaches, bedbugs, and ants, because less insecticide collects on them than on flying insects.

The aerosol method can also be used to apply insecticides to field and gardens crops.[1] Experiments with DDT aerosols produced with methyl chloride have been very successful against the pea aphid. As little as 10 pounds of a 5-per cent DDT solution per acre has given very good control. This is in contrast to 150 gallons of the recommended derris spray (3 pounds of derris [4 per cent rotenone] per 100 gallons). The aerosol method appears very promising for the control of several other agricultural pests, such as the potato aphid, the onion thrips, the peanut thrips, and the Mexican bean beetle.

Plant hormones (15) have also been applied successfully in aerosol form. This method is especially useful to induce the setting of fruit under greenhouse conditions.

Germicides (12), fungicides, or in fact any substances soluble in a liquefied gas can be dispersed in aerosol form by this method.

With the commercial production of aerosols some chemical and physical problems arose. The purification of pyrethrum extract mentioned above was the first to require attention. The water content of the constituents must be kept low to insure against corrosion of the containers. A high grade of material must be used throughout.

The physical behavior of solutions in liquefied gases was also studied. A relationship has been developed to show the increase in concentration

[1] In this work the author is cooperating with F. F. Smith, of the Bureau of Entomology and Plant Quarantine, and L. P. Ditman, of the University of Maryland.

of a liquefied gas solution as the solution is withdrawn from the container (26). The equation is:

$$c = C\left(1 - \frac{Q}{M - VD_g}\right)^{-r}$$

where c = concentration of insecticide after withdrawing Q

C = original concentration
Q = weight of liquid withdrawn
M = weight of initial total contents
V = volume of container
D_g = density of saturated vapor
r = ratio of density of gas to density of solution

A differential manometer method of determining the vapor pressure over a solution in a liquefied gas has been developed, and measurements on several concentrations of oil in Freon have been made. As much as 15 per cent of sesame oil reduced the vapor pressure only 35 mm. from a total pressure of about 5,000 mm.

Densities were also studied with the aid of a small hydrometer bob in a closed system. The bob is calibrated by varying the temperature of pure Freon (26).

Although much progress has been made in the development and practical utilization of aerosols, there is still much research to be done. This method of dispersing insecticides is still very new and considerable research is still necessary. A concerted program is under way to study all angles of this method, especially to provide an inexpensive dispensing unit which will be adaptable for civilian use after the war. The results look very promising.

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CHEMISTRY AT WORK

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The subject of today's paper is "Chemistry at Work." And what is meant by that subject is not a theoretical and involved treatment of the fundamentals of chemical reactions, about which we sometimes write and talk so much and actually know so little; instead let us dwell a little while on chemistry as it fits into the work of every-day life.

New commercial chemical products, such as synthetic ammonia, synthetic alcohols, urea, synthetic rubber, and nylon, are not born in adult maturity into a strange world. Rather they are conceived in abstract form first, as a research laboratory specimen, and they are nurtured through the period of semiworks growth and development until finally a use is found. This is when yesterday's laboratory curiosity becomes a new commercial product for today requiring plant sites, building materials, engineers, chemists, laboratories, and machines.

The beginning is therefore in the research laboratory where trained men and women maintain a relentless quest for truth. How important proper laboratories can be! This building we are in this afternoon, designed and erected through the vision and energy of the man we are honoring today, has provided the physical facilities for training several hundred research men and women in addition to many thousands of undergraduates. The importance of laboratories was concisely summarized by Louis Pasteur in the following words:

"Take interest, I implore you, in those sacred dwellings which one designates by the expressive term: 'Laboratories.' Demand that they be multiplied, that they be adorned. These are the temples of the future—temples of well-being and of happiness. There it is that humanity grows greater, stronger, better."

It is possible that Louis Pasteur wrote better than he realized that day in France, when he called attention to benefits brought to humanity through laboratories. Since that time laboratories have been the birthplace of scores of drugs, health-giving vitamins, and new articles of clothing, food, and shelter that add materially to the beauty and comforts of living. However, twice in a lifetime all of those laboratories, yours and ours, have been turned into instruments for destructive processes rather than constructive. Twice they have been groomed into sinister perfection. In order to prevent its happening again, it is time that men and women throughout the world give thought to better ways of settling international differences before the tools we are forging now destroy us.

The research laboratories so deftly described by Louis Pasteur have played a vital part in developing new materials and processes that lend

themselves to large scale commercial development, thereby providing employment for many more technically trained men and women. The course to be pursued from test tube to tank-car lots is long, but often must be traversed in a few weeks or months. The few crystals, or whatever the new product may be, are first produced in a sufficient quantity, on a semi-works scale, to permit evaluation of such factors as temperature and pressure characteristics, impurity limitations, reaction rates, corrosive effects and thermodynamic data. If the period of semi-works or experimental development can be extended, that is usually helpful in collecting data of direct assistance in design matters. The corrosion data determined by further tests is always helpful in choosing materials with a high degree of selectivity as to permanence in service and tolerance of corrosion products or impurities.

In so restricted a subject as corrosion testing, there are right and wrong ways of proceeding. It is known that in most instances a stressed material will corrode more rapidly than an unstressed material in the same service. It is only reasonable then that in certain corrosive conditions, the test pieces themselves should be stressed, before exposure, to the same degree as calculated for the projected plant equipment. This stressing of metal samples may best be done by the use of electrical strain gauges. These gauges are constructed of thin wires and cemented onto a thin sheet which in turn is attached to the metal part by an adhesive. The wire in the gauges is highly responsive, electrically resistance-wise, to slight increases in length. By standardization, the changes in electrical reaction of the gauge wires, as they undergo slight elongation in conforming to the change in shape of the underlying metal, may be converted directly to strain values. From these strain values, and through knowledge of the elastic modulus of the material used, the stress values are readily calculated.

An even more valuable application of electrical strain gauges has been found in determining the stresses throughout heavily loaded chemical equipment. We might take as an illustration a large smokeless powder press that extends from the basement of the factory building up into the second story. Another example might be a high pressure converter for synthesizing crystal urea by highly compressing two gases, carbon dioxide and ammonia. It is of vital concern to the plant whether the stresses are within a safe operating range or whether some parts of the equipment are bearing an abnormal load that could lead to rupture and possible accident. Fortunately, largely as a result of research in materials of construction and electrical instruments, the newly-developed electrical strain gauges can be depended upon to give reliable information. These tests are made by cementing the gauge units containing the fine wire at points of the equipment to be examined. As many as forty-eight points may be located in one series of resistance circuits, each balanced separately, and recorded on a chart serially for each stress condition as increments of load are applied externally. By this direct means, and at relatively small cost, one is able to construct a complete stress-strain diagram for each

part of the plant equipment where a gauge was attached. These researches in stress distribution in high pressure equipment are expected to have wide application in chemical process post-war developments.

It may be of interest, before leaving the subject of stresses in heavily loaded chemical equipment, to mention a rather simple means of locating, qualitatively, areas of stress concentration. This is done by the use of brittle lacquer, known as "Stresscoat." The lacquer is sprayed on and if dried under controlled conditions will result in a film that will crack at a predetermined degree of strain in the underlying metal. The clarity of the cracks can be improved by the use of dyes. A number of different lacquer compositions, all of high brittleness, provide a range of materials that might justify the term of semi-quantitative in describing the obtainable accuracy.

The problem of corrosion also enters the development of a new commercial chemical product such as nylon.

As you may know, nylon is a long-chain body produced by the reaction between two chemicals such as hexamethylenediamine and adipic acid. These two chemical compounds each have six carbon atoms, so, for convenience and the lack of a better name, the filaments and yarn were called "66" until the name nylon was coined in October of 1938. Actually, quite a number of different nylons are possible. Many already have been made.

In the manufacture of the intermediates in the production of nylon, the reactions are such that corrosion is active, prohibitively so, on many metals. Fortunately, one of the molybdenum-modified stainless steels, if water quenched from 2,000°F., pickled and passivated, is resistant to a remarkable degree. Many tons of this material, valued at $1.00 to $1.50 a pound depending upon size and shape, were used in building the intermediates plant.

Throughout the nylon process development innumerable problems of filtering, heating, agitating, and pumping of liquids, slurries, and viscous polymers were encountered and successfully solved by chemical engineers, several of whom were research specialists on temporary loan.

The selection of construction materials for the nylon plants was the responsibility of a group of engineers with experience and training in one or more such fields as physical chemistry, chemical and metallurgical engineering. One of the more troublesome problems in the nylon development was the selection of material for and the building of metering pumps of high accuracy to operate on molten polymer at 285°C. with no lubricant except the molten polymer itself. In this, there were several problems superimposed on one another, i. e., problems of wear, thermal expansion, heat transfer, fluid flow, and quality of surface finish.

From the first, it was recognized that the smallest variations in process conditions and even traces of any contamination in the nylon polymer were reflected in adverse physical properties in the finished yarn as well as in color characteristics. Each step in manufacture, in semi-works as well as in the commercial plant, had to be scrutinized and standardized

in every detail of design and materials of construction in order that no undesirable side reaction or contaminants would find their way into the material being processed. In this one respect there is a parallel with mankind where undesirable traits of character and temperaments may be transmitted from one generation to another across the narrow biological bridge of a single microscopic cell.

It was a long way between two important events, one the extrusion of the first few inches of a nylon fiber in a physician's hypodermic needle, and the other, the production on a tonnage basis. Yet the steps between these events, although taken at a double-quick pace, were nevertheless orderly and well thought out. For example, a semi-works pilot plant was authorized in January, 1938. On October 27 of the same year, nylon was announced to the world. The first commercial nylon plant, built at Seaford, Delaware, was designed to produce 3,000,000 pounds a year and promptly increased to 4,000,000 pounds a year. Before it turned a wheel, before a pound of material was made, funds were appropriated and the plant was doubled in size to produce a total of 8,000,000 pounds of nylon yarn a year. Now 8,000,000 pounds is a lot of anything, but 8,000,000 pounds a year of nylon is enough to protect a lot of ankles, large and small. Incidentally, this enlarged plant at Seaford has since been duplicated at Martinsville, Virginia, and for the duration of the war the total production of nylon from both sources is pledged to the government for military uses in parachutes, tents, glider tow-ropes, raincoats, and other products.

The story of nylon is a story of invention and initiative, of fundamental research followed by bold development applications based on the premise that a thing or material to be good must be made good for something and produced in quantity and at a cost that will add to the comforts and pleasures of living of the largest possible number of persons. Nylon, like neoprene, synthetic rubber, synthetic camphor, and penicillin, is the result of the system of free enterprise where men can study and work together as they choose, invest their savings, and use their combined capital to initiate larger undertakings, for only in the accumulation of working capital is there assurance of employment. We must not lose sight of the tremendous influence of chemical research which creates new products and new demands and, through that means, employment and general prosperity.

The Development Department of the du Pont Company has made a study of organized industrial research in relation to economic progress as reflected in sales of old and newly developed products. The results from that study indicated that the new projects and products that the du Pont Company will be ready to launch when the war is over, together with outlets for existing products, are expected to bring an all-time high in the Company's peace-time employment. Certainly we are not alone in this prospect.

We thought in 1928 that the products we were selling were modern. We were selling still more in 1942. But note this important fact—in 1942, 46 per cent of du Pont's gross sales consisted of products that either

did not exist in 1928, or were not then manufactured in large commercial quantities. You may safely conclude from these figures, we believe, that industrial concerns are relying upon their research programs to create new products and new jobs, both of which are among the essentials if we are to insure employment and prosperity. What is to be brought out during the coming years in the way of new chemical products is, for the most part, left to speculation. But already products of the research laboratories, possibly only microscopic in quantity, are finding engineering expression in lines of definite length and direction on the drafting boards.

The reason for success of a new chemical venture sometimes is obscure. In the main, it appears to be a combination of factors of supervision, experimental equipment, and the element of time, but perhaps the most important single factor is that of the spirit of cooperation and persistence of the men working on the development. And in speaking of persistence, I'd like to inject at this point a quotation credited to Calvin Coolidge. He said:

> "Nothing in the world can take the place of persistence. Talent will not; nothing is more common than unsuccessful men with talent. Genius will not; unrewarded genius is almost a proverb. Education will not; the world is full of educated derelicts. Persistence and determination alone are omnipotent. The slogan, 'Press On,' has solved and always will solve the problems of the human race."

Nothing will bring better results in solving a particularly hard development problem than intelligent and systematic planning fortified with a determination to keep everlastingly at it. Goethe, the very human German poet, states in summary in his "Faust":

> "He only earns his freedom and existence
> Who daily conquers them anew."
> (Faust, Act V, Scene VI)

Nowhere, we believe, is Goethe's thought more true than in research developments and in application of the results in providing for commercial scale of production.

And now, before closing, it might be worthwhile to say that industry looks to the colleges and universities for men and women of high caliber for their research organizations.

Sometimes while I was teaching at Purdue University, I felt that there might not be quite the community of interest beween the graduate students and faculty on the one hand and the industrial research worker on the other. Once each year this gap appeared to be closed in an exchange of pleasantries when industrial men came to the University to interview candidates for positions. My own feeling is, and I believe it is shared

by many men in industry, that it might be to our mutual benefit to maintain at more frequent intervals, a medium for exchange of ideas and points of view.

It is not uncommon for graduate students or even young faculty candidates in considering industrial positions to express considerable doubt or even a degree of fear at the prospect of leaving—what is called—Academic freedom. From my own experience of having had a professorship for four years at Purdue University followed by approximately fifteen years with the du Pont Company, I definitely recommend considering a career in the chemical industry. The Commencement orators sometimes tell you to get ready to fight the battle of life. Don't you believe it. The life you are preparing for is not a battle; it is simply a succession of rapidly changing situations and if you are smart you will prepare yourself so well that you can adapt to the changing conditions that are bound to come to you in the years ahead. Marie Curie, the daughter of Madame Curie, put a wealth of thought on this subject in two sentences. She said: "There is nothing in life to be feared. It is only to be understood."

You students and particularly the graduate students in chemistry of Iowa State College have been the life concern of Dr. Coover. It will be your responsibility to help to carry forward the standards of quality and accomplishment he has prescribed for you.

FORTY YEARS OF PROGRESS IN FOOD CHEMISTRY

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Since the beginning of the present century food chemistry has not only made great progress but the emphasis has changed. Harvey W. Wiley directed the publication, beginning in 1887, of a series of monographs on "Foods and Food Adulterants." The Food and Drug Act of 1906 was largely a result of his efforts. Without in any way belittling his achievements in the policing of food production and with appreciation of the need at that time for protection of the public from the adulteration, the lack of sanitation and of impurities in foods, the emphasis was negative. The present interest of the food chemist is largely positive. He looks upon his function not so much as protection against unscrupulous or ignorant producers, not so much as protection against hazards, but he thinks of food as a source of positive health.

The food chemist is trained in the related sciences, especially in bacteriology and nutrition, as food cannot be divorced from its use, i. e., nutrition.

Harvey W. Wiley was instrumental in the early development of methods of food analysis which have resulted in a compendium of official methods which have been adopted by the Association of Official Agricultural Chemists.

Forty years ago food chemists were concerned mainly with the determination of the percentages of carbohydrate, protein, fat, and ash of food, that is, its proximate composition. Atwater believed that the chemical composition, showing him the fuel values of all feeds and foods, with a knowledge of the digestibility factors and the energy requirements of man, would make it possible to place the nutrition of man and animals on a sound economic basis. He regarded fruits and vegetables, both high in water, and also eggs and milk, as extravagant food purchases. At that time $0.25 would buy but 645 calories of eggs, but the same $0.25 would provide 8,000 calories from dried beans and 10,000 calories from wheat flour. That was simple nutrition arithmetic. How vast has been the change from this conception of the value of food solely from its yield of energy!

Bulletin 28, first published in 1899 and revised in 1906, was the standard reference until quite recently. Present day tables of food values include more than the percentages of moisture, carbohydrate, protein, fat, and ash, which were given in Bulletin 28. The first considerable division of these general food constituents was made on the ash. Sherman's "Chemistry of Food and Nutrition" was published in 1911. He compiled all available data on food composition and one table of the ash constituents included calcium, magnesium, potassium, sodium, phosphorus, chlorine,

sulfur, and iron. Perhaps it is well that these figures were obtained before the food chemist got so busy on vitamins and amino acids.

Calcium, phosphorus, iron, and iodine are the mineral elements for which care needs to be taken to meet body requirements. The other minerals are easily obtained in even a poorly chosen diet, so that we do not usually consider them in dietary calculations.

PROTEIN: The emphasis on caloric values gave place to interest in protein and it became evident that the quality as well as the quantity of protein was of significance.

Proteins have been of primary interest both at the beginning and at the end of the period covered since 1900. The word "protein" was coined about a century ago from the Greek verb "to take the first place" and was given to what was considered to be the fundamental substance of body tissues.

For two decades overlapping the beginning of the century, T. B. Osborne of the Connecticut Agricultural Experiment Station was analyzing proteins. In 1907 he published a report on the proteins of the wheat kernel and pointed out that these proteins differed so much in the proportions of the then known 16 amino acids they contained that he challenged the prevalent assumption that all proteins were of the same nutritive value.

The question, "How much protein is required?" caused a lively controversy, stimulating observations and experimental study. Voit's standard of 125 grams of protein intake for a man doing moderate work was being challenged. In 1901 Chittenden showed that much less was necessary for maintenance of nitrogen equilibrium and health. He found that from 36 to 55 grams were sufficient for a period of several weeks.

In 1909 Sir James Crighton-Browne asserted that a definite relationship existed between protein intake and racial success, that those on higher protein diets were the more vigorous. This belief is still prevalent and is possibly true, but definite proof, other than surveys, has been lacking. A paper from the Harvard Fatigue Laboratory has just appeared. A diet high in first class protein of 160 grams daily intake and one low in protein, containing little of animal origin and of 50 to 55 grams, demonstrated no effect on muscular efficiency. The experimental period was of only two months duration and on but eight men. Doubtless this question will soon receive further attention.

Studies on animals showed that rations of similar chemical composition were extraordinarily different in nutritive value. A new method of experimentation was developing, the feeding of experimental animals with simple food stuffs and with equivalent chemical substances. In 1906 Hopkins wrote, "No animal can live on pure protein, fat and carbohydrate and, even when the necessary inorganic material is carefully supplied, the animal still cannot flourish." He postulated that many unknown substances were essential, the first guess of the existence of what have since been called vitamins.

At about the same time it became generally recognized that the

values of proteins could not be assessed without consideration and knowledge of the amino acid content. Over the period of the intervening years a vast amount of information has been gained about the amino acid content of foods and some knowledge of the function of amino acids in the bodies of animals. Recently spectacular results based on human studies have been reported by W. C. Rose at the University of Illinois. It has been determined that only eight of the amino acids are required for nitrogen equilibrium and they are called the essential amino acids. It is believed that species of animals differ in these requirements and at least two additional amino acids not necessary for adult life appear to be required for growth.

As recently as 1942 the statement was published in the British Scientific Journal, "Nature," that the classification of amino acids into essential and non-essential is based on studies on the experimental rat and it is doubtful whether there is evidence that any of the known twenty amino acids may be considered to be unessential for man. Thus in less than two years we have reduced the number of amino acids required to be present in the food of man, from 20 to 8. Again we have learned that the rat is not a human being.

A rather startling finding has been the rapidity with which a deficiency of certain amino acids becomes evident. Nine days deficiency of valine resulted in loss of appetite and in fatigue and restoration produced an almost immediate effect. Vitamin deficiencies are not as dramatic.

Amino acids have been tagged with heavy nitrogen to trace their route and deposition in body tissues, showing an astounding rapidity of change in the amino acid and protein structure of the body.

Proteins are vitally important nutritionally but also economically. The cheaper vegetable proteins are being examined as possible substitutes for the more expensive animal proteins. Processing of vegetable proteins through a bovine or poultry factory is expensive and inefficient.

The rat is being displaced by the "despised" microbe as a means for measurement of amino acids. Only within the last year has it been possible to separately determine the three acids, valine, leucine, and isoleucine, and this has been done by means of microbial methods.

All enzymes so far studied and many hormones are proteins. A virus causing a disease of the tobacco plant, known as tobacco mosaic, has been identified as a protein. Dr. Paul Cannon of the University of Chicago states that all bacteria causing infections are foreign proteins. He maintains that, since amino acids are essential in the development of antibodies, reduced protein intake may seriously impair ability to resist infections. This is of especial interest in control of plagues in war areas and in depletion of body tissue from disease or surgery. The ability to form antibodies is impaired and the diagnosis of an infection as the cause of death is really giving first place to a secondary factor.

Protein is as necessary in the formation of hemoglobin as is iron.

Proteins broken down by enzymatic action to form "protein hydrolysates" are being used parentally, instead of the more expensive and diffi-

cult to obtain amino acids, post-operatively, for war wounds, and after severe burns, as in the cases from the much publicized Cocoanut Grove disaster.

The knowledge gained in the last two years about amino acids is making possible more intelligent supplementation of inadequate proteins, especially the more efficient use of vegetable proteins. However, we do not yet know much about the amino acid content of vegetable proteins.

IRON: Another illustration of a changed concept is that of iron. In Sherman's first edition of "The Chemistry of Food and Nutrition," 1911, fifteen pages were devoted to the controversy of organic vs. inorganic iron and the biological utilization of iron.

In 1902 Bunge, in Germany, published a series of experiments on rats, rabbits, and dogs in which he showed that a diet of milk and rice alone, or milk, rice, and ferric chloride, resulted in highly anemic animals. Contamination of the iron salt with copper would have changed the result. This study led to the belief that even though inorganic iron and organic iron followed the same route in the body, hemoglobin was derived essentially from organic iron. Now we know that inorganic iron salts, with small amounts of copper, are effective sources of available iron. We need much more information about the availability of the various forms and sources of iron.

INTERRELATIONSHIPS: The influence of copper on the availability of iron illustrates the biological effect of catalysts. It is not surprising that catalysts operate in the field of biological chemistry. Only within the last few years have we learned much about the subjects of biological catalysts, trace elements in foods and in the animal body, about vitamins and enzymes and about their interrelationships and interdependence.

Now the interrelationships and effects of various nutrients upon each other are filling the literature. To mention only a few, cobalt deficiency on the anemia of cattle, carbohydrates on the requirements for thiamin, influence of choline on the utilization of vitamin D, of copper in food processing equipment on ascorbic acid, mineral oil on availability of vitamin A, the relationship of ascorbic acid and of protein to infections, of protein on calcium absorption, of temperature of environment on the thiamin, pyridoxine and choline requirements of man, and of intense sunlight which increases the need for vitamin A, riboflavin, and niacin. These few examples illustrate a variety of types of interrelationships and many of them are similar to reactions in other fields of chemistry. To mention one more example, we do not yet know the effects of imbalance caused by deficiencies or of excesses of certain nutrients.

The early food chemist visualized the substitution of pills for food. The druggist now has that idea, but the modern nutritional chemist, as he learns more about the complexity of the apparently simple matter of eating, believes ever more firmly that food is essential.

COLLOID CHEMISTRY: A new development is in the field of the applications of colloid chemistry to food chemistry. A few examples will serve to illustrate the applications of the principles of colloids to cookery processes and procedures.

The gluten of flour has colloidal dimensions. Gluten particles of cake and pastry flours are of smaller size and more dispersed than those in bread flours, a quality of use in obtaining the desired texture in cakes. The same principles in the control of time, temperature, concentration, and various manipulations are effective in the making of a cake as in reproduction of other colloids. A paper at the last meeting of the Cereal Chemists was on the effect of varying mixing speeds and dry milk solids in the making of bread.

Heating and homogenization of milk in the manufacture of evaporated milk result in greater dispersion of the fat. But heating of protein sufficiently to produce coagulation decreases the dispersion.

The addition of acids or alkalies in strengths insufficient to cause true chemical change may either increase or decrease the dispersion of particles.

Mayonnaise is an emulsion of the oil-in-water type composed of salad oil, eggs, vinegar, and spices. In the manufacture of margarine, while the margarine oil and the milk are being churned together, the emulsion is of the reverse to that of mayonnaise, water-in-oil.

SOME CONTRIBUTIONS OF THE FOOD CHEMIST: Much of our present common knowledge, many carefully controlled commercial procedures resulting in foods widely and generally available, have been developments since 1900.

The study of the bacteriology of canning started in 1895, when Prof. H. L. Russell applied bacteriology to trouble with swells encountered by Wisconsin pea canners. By about 1924 adequate processing procedures, based on extensive bacteriological studies, had been established, resulting in the safe canned foods industry as we know it. Methods were devised for measuring the heat penetration in sealed cans, to obtain data on the heat transfer in different foods with varying media and in different sized cans. The recommended processes are sufficient to assure in the center of the can the thermal death point of *Clostridium botulinum*, a widespread, dangerous, and resistant organism. Since 1925 no case of poisoning from the toxin produced by this organism has occurred in any commercially canned food packed in this country. This has been a notable industry achievement. Other industries can point with pride to similar accomplishments.

Just before 1900 Dr. Samuel Prescott determined that spoilage in canned foods was microbial in origin. It is surprising that this fact was not established until ninety years after Appert, during the Napoleonic Wars, had first succeeded in preserving food by means of heat in a sealed container. It was fifty years after Appert that Pasteur introduced the principle of pasteurization. The advancements made in the last forty years have been truly astounding.

A few examples of the contribution of the chemist to new developments in foods are the following: cereals of the rolled, flaked, granulated, and pre-cooked, ready-to-eat types; good milk produced under sanitary conditions either as raw, pasteurized, evaporated, or dried milk; the application of bacteriology to all foods with reference to control of spoilage and food poisoning; the modern meat industry; a new industry of edible

fats and oils from cotton and other seeds; spray drying of eggs; development of sanitary unit packaging, which in some cases involves methods and packaging which will delay the development of rancidity; quick freezing of a wide variety of foods; commercial dehydration of foods where an old home industry was commercialized during the Boer War and subjected to scientific study during World War I, greatly improved during World War II. We now have a food industry, largely developed since the beginning of the century, with a wide variety of foods from soup, both in cans and dried, to shelled nuts in air tight paper containers.

Electronics is just now being applied to sterilization of foods in packages.

FLAVOR IN FOOD: We have learned much about the action of enzymes in foods on their qualities after processing and storage. Both canned foods and frozen foods are subjected to a blanch before canning or freezing. This inhibits enzyme activity, preserving color and in the case of frozen foods, prevents a hay-like off-flavor which develops in frozen vegetables if not adequately blanched.

Flavor in food is one of the newer interests of the food chemist. Flavor is still quite largely an "unknown" in food chemistry.

There are not yet objective tests for variations in flavor. Some chemical tests parallel loss in flavor. Ascorbic acid loss usually coincides with loss of quality, which in turn is closely akin to loss of flavor.

Flavor is a mixture or combination of sense reactions, emotional rather than chemical. The sight or appearance of food, odors, texture, and memory are all of influence in determining individual flavor preferences. People vary in all of these responses, as anyone who has used a taste panel can testify.

A recent paper carries the title, *Relative Taste Potency of Some Basic Food Constituents and Their Competitive and Compensatory Action.*

At the meeting of the American Chemical Society this fall, one session was devoted to the subject, *Food Flavor and Quality.* A few of the titles of papers at that meeting were the following: *Some Visual Aspects of Quality Control and Quality Research; Physico-chemical Research and Its Relationship to Flavor Development and Control; Volatility in Food Flavor; Flavor in Food Fats* (i. e., enhancement of flavor is desired in some fats and its elimination in others); *The Problem of Prevention of the Development of Oxidative Changes.*

To quote from one paper: "Preservation of the hidden qualities of foods is in a very primitive state of development. Finer hidden qualities, not readily apparent to the consumer, can be bred into fruits and vegetables and improved methods of agriculture and transportation and food processing will eventually preserve most of the nutritive properties for the ultimate consumer." The as yet undeveloped hidden qualities are of great interest to the food chemist because of the possibilities of improvement of foods, especially the processed foods.

The term "processed foods" should include certain raw foods. Some

so-called "raw foods," as fresh fruits for example, have passed through six or eight-unit operations requiring elaborate equipment and technological skill.

Some of the procedures which have been developed to enhance appearance or flavor are now being subjected to assay for the effects on nutritive values.

EFFECTS OF WAR ON THE WORK OF THE FOOD CHEMIST: As previous wars have stimulated the production of foods suitable for the use of armed forces, so have the two wars within this century. New foods have been devised to meet specific requirements, as butter which will withstand the high temperature of the tropics by the addition of a cotton seed oil preparation. Heat does not cause the development of rancidity in this product, as it does with butter, although the flavor is adversely affected. The dehydrated food industry received a great impetus because of the needs of transportation in a world-wide war. We have been learning which foods can be more successfully dried and we have found out considerable about the packaging and storage problems of that industry.

Concentrated rations supplying the minimum nutritive requirements and suitable for combat troops have been developed and are being improved. Knowledge about human food requirements, practically unknown during World War I, has made these concentrated rations possible.

The army and navy had need for more information about the nutritive values of foods, especially the vitamin content of foods as eaten. Until recently, most of the tables giving nutritive values included few data except on raw foods. One result of this demand, because of the large amounts of canned foods used, was the initiation of a long-time extensive study by the canning industry on the nutritive value of canned foods. The first series of papers has just appeared in the *Journal of Nutrition*.

Certain procedures used in modern canning practice are favorable to retention of some of the nutrients. The virtual "exhaustion" of air, by heating the food before sealing the can, has a favorable effect on the retention of vitamin C. While losses of soluble vitamins and minerals take place during blanching, those dissolved in the liquid in the can combined with what is in the vegetable or fruit, furnish more than in the usual methods of home cooking of raw foods. Methods of preparation of canned foods for serving will be a phase of this study. If the general public can be educated to use the information which is being made available, the effects on the nutrients obtained will be stupendous. Discarding the liquid in the can, or for that matter the water in home cooking of vegetables, sends nearly half of some of the food values down the kitchen drain.

Studies are in progress on the effects of environmental temperatures of canned foods during storage. In temperate climates it has been found that storage does not cause serious losses of the essential dietary constituents in canned foods. Until more information is available the army believes that in hot and humid climates, the fortification of certain foods

is desirable if not necessary. Requirements have been imposed for stability of both natural and fortified foods at 100°F. for one year, far more severe than domestic requirements.

This canning industry study is an extensive piece of work and already has supplied a considerable amount of information, especially on the ranges of vitamin and mineral content which reasonably may be expected to occur. It will show the proportions of the available canned foods giving close to average values and the procedures which result in extremely high or low content.

Of practical value to all of us as consumers will be the improvements which may be anticipated in the procedures used in canning, the growing of crops not only for good yields per acre but higher nutritive values, and also in the education of people in the preparation of canned foods for serving.

We are so familiar with the present tin can that it may be surprising to realize that the first open top sanitary style can appeared in 1900, to replace the former solder sealed can. It was not universally used until about 1918. The needs of global warfare and the shortage of tin for food containers has resulted in improved packaging of foods in paper containers. The shipping carton of today is a vast improvement over that used during the first few months of the war. Plastics and aluminum alloy cans are already familiar to you. However, no one has yet been able to put a zipper on the can.

The Quartermaster Corps has stimulated work on anti-oxidants. The anti-oxidants being used have shown variability at different temperatures. Certain ones which have proved to be fairly satisfactory at the boiling point of water are of little use at room temperature; others which are effective at room temperature are inactivated by heat. Some of these reactions show sharp changes at certain temperatures.

Fortification of Foods: The enrichment and the fortification of foods is another phase of the work of the food chemist. Aside from the question of the justification or the desirability of the practice, there have been practical problems. The stability of white flour, over long periods of storage, has been the principal reason for its general use and acceptance. The enrichment of flour has not restored the values removed in milling, and doubtless improvements will be made both in nutrients added to flour and in stabilizing these substances.

One of the substances added to enriched flour is iron. Not only the stability of the various forms of iron used in the fortification of foods, but the availability of the iron to the body and the stability of the food itself in the presence of ionizable iron are problems still under investigation.

Both food chemistry and nutrition have been builded upon a scientific foundation. This fact has probably had at least some effect in safeguarding us as a people from fortification of almost everything we eat as a result of overenthusiasm. White flour and cereals have been generally accepted as justifiably proper mediums for fortification at least back to pre-processing levels or natural levels of dietary essentials. At present

the enrichment or fortification of the following foods is favored: flour and bread, possibly cornmeal, the vitamin D fortification of milk, vitamin A to fats, and iodine to salt. The Foods Committees of the American Medical Association and of the National Research Council oppose the fortification of carbonated beverages and confections or the fabrication of foods or beverages containing multiple fortification to the extreme point of provision of all nutrients necessary for the day in one or two servings of a single food.

The education of the public on methods of preparation of natural or commercially prepared foods and on the desirability of the use of foods instead of super-fortified foods or concentrates certainly is more desirable than uneconomical fortification. Dr. Elvehjem has commented that the public seems determined to spend about so many millions at the drug store. Perhaps less harm and just possibly some good may result if it is spent on vitamin pills instead of the old fashioned patent medicines.

THE NUTRITION FOUNDATION: A recent, entirely new development is that of food manufacturers cooperatively providing for fundamental and basic research. The Nutrition Foundation is financed by forty-two commercial food companies for the purpose of allotting grants to laboratories in educational institutions. The research is largely fundamental in character, although a few grants have been made in response to requests from the army for information of immediate interest. Individual firms are visualizing something beyond the confines of their immediate interests, their obligations to develop fundamental research in food and nutrition. This recognition does not ignore much fundamental research which has been conducted by many firms and industries, but it is a new approach. It is a union of industry and research, something which would not have been possible only a few years ago.

COMMENTS ON FUTURE TRENDS: Perhaps it is unwise to speculate on the future, because time eventually catches up with prophesy like it does with politicians, but it is interesting. It is especially so in a field where the progress made since the beginning of the century has been as rapid and as great as it has in the realm of food chemistry.

It would seem inevitable and desirable that the science of food chemistry will become increasingly allied with and interdependent on nutrition as it has been in the studies on proteins, on bacteriology as it has in the canning industry, on engineering in the development of equipment, and with other phases of chemistry such as physiological chemistry and enzyme chemistry.

The fundamental scientific research in these interrelated sciences will stimulate and will give a sound foundation for an increasing amount of technical and applied research.

Economic phases will also enter into the development of industrial applied food chemistry. As George A. Sloan recently said at the Institute of Food Technologists, "Only by the development of products acceptable to the public, can companies expand to make employment and thus make a powerful contribution to prosperity and a higher standard of living."

This development must be economically sound, by improvement of existing products, fabrication of new products, and, through mechanical means, reduction of production costs.

We do not yet know many things we should about constituents of the foods we eat, their nutritive values under various conditions, the interrelationships of the various nutrients, the conservation of essential nutrients by better processes of production and preservation. Much needs to be learned about packaging from the angles of food chemistry, nutrition and protection of food flavors.

Food chemists will discover uses for food stuffs other than as nutrients for men and beasts, especially in an era of surpluses. Foods may be sources of hormones, substitutes for gasoline and other fuels, in ways somewhat similar to the manufacture of plastics and the casein-base materials, as medicinals, and for many other possibilities as yet unthought of.

The food chemist is interested in supplies of foods, therefore in the problems of farm production and the transportation of foods. A very large proportion of our population is directly concerned with the production of food, its manufacture, processing, packaging, distribution, and ultimate uses, and more and more will the problems of these groups be presented to the food chemist and technologist.

And finally, the food chemist in the phases of food research bordering on nutrition is concerned with the public health aspects of food, in what food is needed as well as in what will sell. Since 1900 the emphasis has changed from the negative to the positive, to the production of better foods of higher nutritional values and in all probability this trend will be further accentuated.

CONCLUDING COMMENTS: There has been no attempt to make this in any way a complete or even a partial survey of the progress of food chemistry during the past forty-four years, but merely to give a few developments which might be of interest to this group.

The more narrow confines of the chemistry of foods have been enlarged to include or cooperate with other phases of chemistry and of bacteriology and particularly is there evidence of the union of food chemistry and nutrition.

Illustrative of the extended influence and interest in the field of food chemistry is the fact that in 1900 there were only a mere handful of persons engaged in this work. Now, literally hundreds gather at the meetings of the groups and associations including food chemists among their members. The development of food chemistry as a science and its applications in the food industry are due to many factors, among others the growth of the related sciences and to wider interest in nutrition.

Food chemistry is no longer a "closed corporation" or exclusive fraternity. This is fortunate, for our interests are food and human-wide.

SYNTHETIC RUBBER

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The development of synthetic rubber is based upon the wealth of information which has been accumulated on the structure and properties of natural rubber. According to the present concept, natural rubber is a linear polymer of extremely high molecular weight, in which isoprene is the recurring unit. Isoprene was shown to be present in the products resulting from the pyrolysis of rubber by Williams in 1860. In 1875 Bouchardat succeeded in reconverting isoprene to a solid rubber-like material by polymerizing it with the aid of concentrated hydrochloric acid. However, the study of a means of preparing synthetic rubber from isoprene never reached appreciable proportions. In fact, no known polymer of isoprene has been synthesized to date which has properties that can compare with those of natural rubber, although the Russians, shortly before the start of their present war with Germany, were reported to have developed a new method for making isoprene rubber (1). From a commercial standpoint, no known economical source of isoprene has been made available. The Germans in the period following the first world war turned their attention to butadiene in preference to other diolefins.

The first synthetic rubber of note was prepared from dimethylbutadiene, which had been found to polymerize to a rubber-like mass in 1900. During World War I, the Germans developed this type of rubber on a commercial scale. It is reported that they operated a plant with a capacity of 150 tons per month for a considerable period. Methyl rubber, as the product was called, was an inferior grade of rubber, and as a result its manufacture was discontinued at the close of the war. It was prepared from acetone by the following series of reactions:

$$2\,(CH_3)_2CO + 2H \rightarrow (CH_3)_2COH\cdot COH\,(CH_3)_2$$

$$(CH_3)_2COH\cdot COH\,(CH_3)_2 - 2H_2O \rightarrow CH_2 = \overset{CH_3}{\overset{|}{C}} - \overset{CH_3}{\overset{|}{C}} = CH_2$$

$$x\,(CH_2 = \overset{CH_3}{\overset{|}{C}} - \overset{CH_3}{\overset{|}{C}} = CH_2 \rightarrow \left[- CH_2 - \overset{CH_3}{\overset{|}{C}} = \overset{CH_3}{\overset{|}{C}} - CH_2 - \right]_x$$

The polymerization of the monomeric diolefin was effected by using sodium as a catalyst or by heating at about 70°C. In an alternate method, the polymerization was allowed to proceed at room temperature, after the monomer had been seeded with polymer.

During the late 1920's and the early 1930's, the Germans developed a

group of products known as the Buna rubbers. The first ones of the series were prepared by polymerizing butadiene with sodium. The name Buna is derived from the first two letters of Butadiene in combination with the symbol Na for sodium. It is postulated that the sodium adds 1,4 to the diene and that the resulting organometallic compound adds 1,4 to another molecule of diene. There is evidence that some 1,2-addition also occurs. The addition process is thought to continue until a long chain molecule is built up. Two types, Buna-85 and Buna-115, were developed. The numbers designating the types refer to the molecular weight in thousands.

In 1931, du Pont announced a new synthetic rubber which was called Duprene. This name was later changed to Neoprene. Neoprene, a polymer of chloroprene, was developed by Carothers as an outgrowth of the work by Nieuwland on the polymers of acetylene.

Thiokol was introduced commercially in 1932. This class of rubbers consists of a series of organic polysulfides developed by Patrick.

In 1935 the commercial production of the Buna rubbers was announced by Germany. Emulsion polymerization replaced the older method involving the use of sodium; and copolymers, known as Buna S and Buna N, replaced the polymers previously prepared from butadiene alone. Synthetic rubbers of the copolymer type are generally prepared from a diolefin and an unsaturated compound of the vinyl type. Thus, Buna S is a copolymer of butadiene and styrene, and Buna N is a copolymer of butadiene and acrylonitrile. The product is a true copolymer only if both species of monomer enter into the formation of the individual molecules which make up the polymerizate.

Butyl rubber was discovered in 1937 and announced to the public by Standard Oil Company (N.J.) in 1940. A pilot plant was in operation at the time of the announcement. In June, 1941, the construction of a Butyl rubber plant with a capacity of ten tons per day was authorized. In October of the same year, this capacity was doubled. Goodrich and Goodyear also announced in 1940 synthetic rubbers under the names of Ameripol and Chemigum, respectively. By April of 1941, the Standard Oil Company of Louisiana had completed construction of a Buna N plant with a capacity of five tons per day.

Thus, at the time of Pearl Harbor, the foundations of the synthetic rubber industry in the United States had already been developed. Without the wealth of information available at that time, it is doubtful that the amount of synthetic rubber recommended by the Baruch Committee could have been made available in time to meet the requirements of the national emergency. It should be noted, however, that in spite of the information available at the time of Pearl Harbor, a tremendous amount of effort had to be expended on research and development of raw materials, manufacturing processes, and on the fabrication of synthetic rubber into finished articles. Research is still in progress on a very large scale in these fields. At the present date, the rate of production of synthetic rubber has reached a figure in excess of our imports of natural rubber prior to the war. Production figures for Buna S, Butyl, and Neoprene GN are given in Table 1.

TABLE 1
PRODUCTION OF SYNTHETIC RUBBER (2) (LONG TONS)

Type Rubber	Rated Annual Capacities			Estimated Ultimate Annual Capacity with Present Plants U. S. and Canada	Estimated Annual Rate 4th Quarter 1944 U. S. and Canada
	Baruch Recommendation U. S. Only	Present U. S. Only	Present U. S. and Canada		
Buna S	845,000	705,000	735,000	1,000,000	780,000
Butyl	132,000	68,000	75,000	75,000	38,000
Neoprene GN	69,000	63,000*	63,000*	70,000	57,000
Thiokol N	60,000	(Program Suspended)			
Total	1,106,000	836,000	873,000	1,145,000	875,000

* Includes 14,000 tons plant capacity scheduled for completion during 1944.

Thiokol N was at one time included in the synthetic rubber program for retreading and for passenger tire production. However, it was later found that conservation methods and availability of reclaimed rubber were sufficient to meet the situation without the production of the 60,000 tons per year recommended by the Baruch Committee (2). Buna N is not included in the above table. The best unofficial estimates place plant capacity in the United States at 29,000 to 30,000 long tons per year (3).

Real progress in the development of synthetic rubber was not realized until the idea of synthesizing natural rubber was abandoned.[1] As soon as the emphasis was placed on making synthetic rubber by the polymerization of the many types of available unsaturated compounds, polymers were produced which began to excel natural rubber in one or more respects. Recently, phenomenal progress has been made and is still being made in the construction of tires from synthetic rubber. For many other uses, synthetic rubbers have properties that excel those of natural rubber. However, it must be admitted that so far no synthetic has been developed that is equivalent to natural rubber in all respects. Most types tend to build up more heat under flexing conditions than does natural rubber. For this reason and as a result of problems in adhesion, it has been difficult to produce a synthetic fully the equal of natural rubber in the fabrication of tires.

The preparation and properties of the more important types of synthetic rubber are discussed below.

Buna S

Buna S constitutes the bulk of our synthetic rubber program, since it appeared to be the most promising type that could be made available in quantities sufficient to meet the emergency requirements of the tire

[1] The writer, however, is of the opinion that the incorporation of more isoprene into synthetic rubbers or the synthesis of an all-isoprene rubber may lead to improved products which more closely approach natural rubber in their properties.

industry. The butadiene and styrene required to produce it are prepared or can be prepared by the following methods:

TABLE 2

PREPARATION OF BUTADIENE AND STYRENE

Butadiene

1. $C_2H_5OH \longrightarrow CH_2=CH-CH=CH_2$
2. $n-C_4H_{10} \longrightarrow CH_2=CH-CH=CH_2$
3. $n-C_4H_8 \longrightarrow CH_2=CH-CH=CH_2$
4. Naphtha $\longrightarrow CH_2=CH-CH=CH_2$
5. $HC\equiv CH+H_2O \longrightarrow CH_3CHO$
 $2CH_3CHO \longrightarrow CH_3CH(OH)CH_2CHO$
 $CH_3CH(OH)CH_2CHO+2H \longrightarrow CH_3CH(OH)CH_2CH_2OH$
 $CH_3CH(OH)CH_2CH_2OH \longrightarrow CH_2=CH-CH=CH_2+2H_2O$
6. $C_2H_5OH \longrightarrow CH_3CHO+H_2$
 CH_3CHO as in (5) $\longrightarrow CH_2=CH-CH=CH_2$

Styrene

1. $C_6H_6+C_2H_4 \longrightarrow C_6H_5C_2H_5$
2. $C_6H_5C_2H_5 \longrightarrow C_6H_5CH=CH_2$

Butadiene can also be prepared from butylene glycol-2,3 obtained by fermentation.

In the manufacture of Buna S, styrene and butadiene are emulsified in water with the aid of an emulsifying agent, and the polymerization is effected with a peroxide type of catalyst. Approximately 25 per cent of styrene is used. Modifiers for the polymerization, such as thiuram disulfides, mercaptans, zanthogen disulfides, etc., have been used (4). These modifiers favor the formation of linear polymers in preference to the undesirable branched and cross-linked types of polymer.

TABLE 3

PREPARATION OF BUNA S

Polymerization

$$xC_4H_6+C_6H_5CH=CH_2 \longrightarrow \left[(-CH_2-CH=CH-CH_2-)x\ -CH_2-\underset{\displaystyle C_6H_5}{CH}-\right]_n$$

Typical Reaction Mixture		Reaction Conditions
Butadiene	60–75 parts	10–15 hours at 40–60° C.
Styrene	40–25	
Emulsifying agent	1– 5	
Catalyst	0.1–1.0	
Modifying Agent	0.1–1.0	
Water	100–250	

Buna S is compounded and fabricated in a manner similar to natural rubber, although variations in the formulae are used in order to bring out

its optimum properties. Buna S possesses good abrasion resistance, good age resistance, and wears well in tire treads. It is deficient under conditions of hot service, wherein a change takes place known as "heat embrittlement."

Perbunan

Perbunan, formerly called Buna N, is a copolymer of acrylonitrile and butadiene. It is synthesized by the emulsion process in a manner analogous to Buna S.

TABLE 4

PREPARATION OF PERBUNAN AND RAW MATERIALS

Acrylonitrile

1. $HOC_2H_4Cl + NaCN \longrightarrow HOC_2H_4CN$
 $HOC_2H_4CN \longrightarrow CH_2=CHCN + H_2O$
2. $(CH_2)_2O + HCN \longrightarrow HOC_2H_4CN$
 $HOC_2H_4CN \longrightarrow CH_2=CHCN$
3. $CH_3COO\,C_2H_4CN \longrightarrow CH_2=CHCN$

Butadiene

(See Table 2)

Polymerization

$$xC_4H_6 + CH_2=CHCN \longrightarrow \left[(-CH_2-CH=CH-CH_2-)x\ -CH_2-\underset{\displaystyle CN}{\underset{|}{CH}}- \right]_n$$

About 25 per cent of acrylonitrile and 75 per cent of butadiene are used in the polymerization process. Higher amounts of the nitrile increase oil resistance, but brittleness at low temperature is adversely affected. The polymer during the early stages of the polymerization is oil-like in nature, and the polymerization as in the case of Buna S must be stopped at an optimum conversion in order to prevent the product from becoming too hard and tough, which it will, if the conversion is allowed to proceed too far. Polymerization modifiers are used as in the Buna S synthesis. It is indicated in Table 4 that the butadiene polymerizes by 1,4-addition. However, there is evidence that some 1,2-addition also occurs.

Perbunan is a highly oil-resistant type of rubber. It has good to excellent resistance to abrasion, tear, cold and hot flow, aging, and gas diffusion; but its resistance to checking in sunlight is only fair. Like all synthetics it generates appreciable heat through hysteresis. However, it can be used at higher temperature levels than natural rubber. Due to its oil resistance, it is used in hose for transferring hydrocarbons, in self sealing so-called bullet-proof gasoline tanks, and in many other applications where rubber comes in contact with oil.

The copolymers of butadiene with acrylonitrile or with styrene are superior to the straight polybutadiene type of polymer. They are far more rubber-like, and possess better processability and tensile strength than their straight butadiene analog.

Neoprene

Neoprene is a polymer of chloroprene. It also is prepared by the emulsion technique.

TABLE 5

PREPARATION OF NEOPRENE AND RAW MATERIALS

Chloroprene

1. $2HC\equiv CH \xrightarrow[NH_4Cl]{Cu_2Cl_2} CH_2=CHC\equiv CH$

$CH_2=CHC\equiv CH + HCl \longrightarrow ClCH_2-CH=C=CH_2$

$ClCH_2-CH=C=CH_2 \xrightarrow{\text{Isomerization}} CH_2=CH-CCl=CH_2$

Polymerization

$xCH_2=CCl-CH=CH_2 \longrightarrow \left[-CH_2-\underset{Cl}{C}=CH-CH_2-\right]_x$

Chloroprene is stated to polymerize 700 times as fast as isoprene. Since the polymer does not keep well in the raw state, it must be vulcanized within a reasonable time after preparation. The method of vulcanizing polychloroprene is quite different from that used with natural rubber and other synthetics discussed so far. Metallic oxides such as magnesium oxide are used in place of sulfur, although sulfur is stated to act as an accelerator for the vulcanization.

Neoprene is comparable to natural rubber in many respects and is superior in its resistance to the solvent action of petroleum products and natural oils. It possesses good to excellent resistance to abrasion, sunlight, aging, heat, and flames. Because of these properties, neoprene has found extensive use in hose for handling hydrocarbons, gasoline tank linings, cable coverings, water-resistant clothing, and oil-resistant electrical insulation.

Butyl Rubber

Butyl rubber as produced commercially today is a copolymer of isobutylene and isoprene.

TABLE 6

PREPARATION OF BUTYL RUBBER AND RAW MATERIALS

Isobutylene

Cracking of Petroleum Fractions $\longrightarrow (CH_3)_2C=CH_2$

Isoprene

Cracking of Petroleum Fractions $\longrightarrow CH_2=\underset{CH_3}{C}-CH=CH_2$

Polymerization

$$x(CH_3)_2C=CH_2 + CH_2=\underset{CH_3}{C}-CH=CH_2 \rightarrow \left[\left(-\underset{CH_3}{C}-CH_2\right)_x -CH_2-\underset{CH_3}{C}=CH-CH_2-\right]_n$$

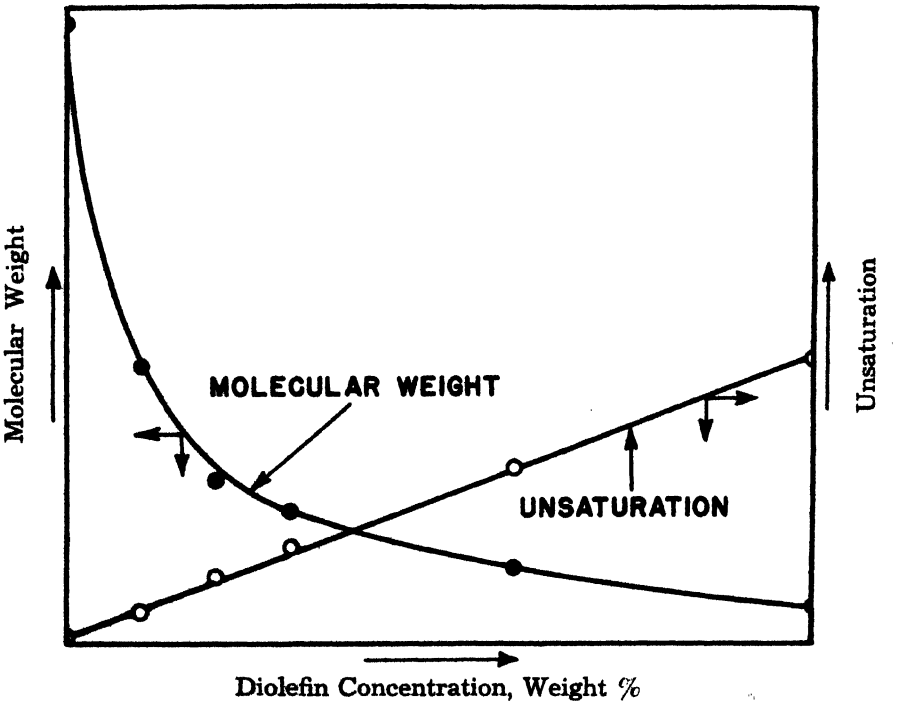
Which end of the isoprene unit is joined to the methylene group in the isobutylene unit is not known, but it is known that the isoprene polymerizes by 1,4-addition. No products of ozonolysis corresponding to an unsaturated side chain have been detected (5).

Butyl rubber is made by a continuous polymerization process which leads to the formation of a polymer of rather uniform molecular weight. The polymerization is effected below —100°F. This low temperature is necessary since higher temperatures lead to low molecular weight polymers, which lose their rubbery properties and eventually become oily in nature as the temperature is increased. The reaction, which is catalyzed by metallic halides of the Friedel-Crafts type, is extremely rapid.

Other diolefins such as butadiene, piperylene, dimethylbutadiene-2,3, etc., can be used in place of isoprene. Since a minor proportion of diolefin is used, the resulting polymer has only about 1 to 3 per cent of the unsaturation of natural rubber. In spite of this low unsaturation, the uncured polymer is susceptible to oxidation and must be protected with a stabilizer.

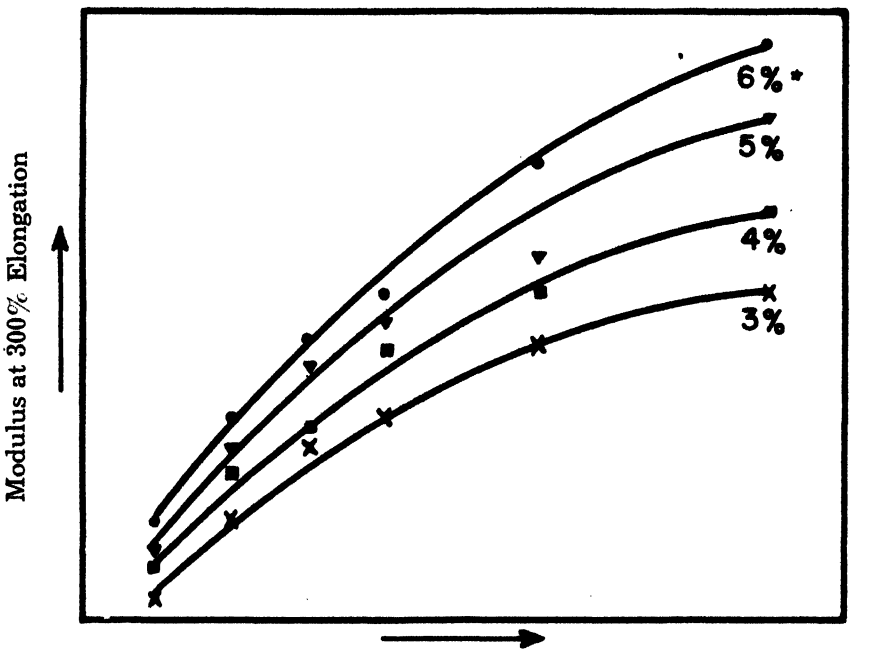
At any given reaction temperature the quantity of diolefin in the polymerization mixture affects the properties of the polymer. Unsaturation increases and molecular weight decreases as the diolefin content is increased.

FIGURE 1
EFFECT OF DIOLEFIN CONCENTRATION



The quantity of diolefin also affects the modulus, i.e., the "tightness," of the cured rubber. The greater the amount of diolefin in the polymer, the higher the modulus.

FIGURE 2
MODULUS OF BUTYL RUBBER (TREAD STOCK)



* Per cent of Diolefin in the feed.

Butyl rubber is compounded and cured in much the same way as natural rubber, except that ultra-accelerators are required due to the low unsaturation. Butyl, Neoprene, and natural rubber are the only rubbers that possess a high pure gum tensile strength. All other well known synthetics have low gum tensiles and must be compounded with carbon black in order to realize useful properties. Both Butyl and Neoprene may be used either in pure gum or carbon black formulations.

Since Butyl is an all hydrocarbon polymer, it is not oil-resistant, being similar to the natural rubber in this respect. As a result of its low unsaturation, Butyl is quite resistant to chemicals such as mustard gas. Some modifications are resistant to strong inorganic acids and even to ozone.

An outstanding characteristic of Butyl is its impermeability to gases. Inner tubes made of Butyl are reported to retain their air pressure six to ten times as long as natural rubber. In a recent road test, natural rubber inner tubes lost air eight times as fast as Butyl inner tubes that were run in the same test. During the eleven weeks period covered by the test, the

former had to be reinflated approximately every two weeks, whereas the Butyl tubes did not require reinflation.

To date the major uses of Butyl rubber have been in inner tubes and in proof goods, i.e., impregnated fabrics. Part of the inner tube production of the United States is being rapidly converted to Butyl rubber, and total conversion is a possibility as soon as sufficient Butyl is available. Although Butyl does not fit into the present tire program, tires have been built of Butyl that have run up to 25,000 miles at speeds not in excess of 40 miles per hour. No natural rubber was used in the construction of these tires.

Thiokol

Thiokol is the reaction product of sodium tetrosulfide and adihalide such as ethylene dichloride or B,B′-dichlorodiethyl ether. The polymer, although it contains no olefinic linkages, can be cured with metallic oxides such as zinc oxide. This type of synthetic rubber possesses excellent oil resistance. As a result, it is used in hose for handling petroleum products and in gaskets.

Norepol

A new type of synthetic rubber called Norepol has recently been developed. Commercial varieties under the trade names of Agripol (6) and Vulprene (7) have been introduced on a limited scale. Oils of the non-mineral type, such as soybean oil, furnish the basic material used in the synthesis of this type of rubber. By special methods, the unsaturated fatty acids can be isolated from these oils. They are converted to polymers which have the properties of dibasic acids. These dibasic acids freed essentially of monomeric acids (6) are esterified with dihydric alcohols to form high molecular weight products which are capable of being vulcanized (7).

The commercial varieties have tensile strengths of the order of 400 to 1200 pounds per square inch. Norepol itself, when prepared from purified fatty acids, possesses tensile strengths in the range of 1000 to 2000 pounds. The future commercial possibilities of this type of rubber are as yet unknown. It has been reported that the biggest single use for Agripol is in gaskets for food closures (6).

No attempt has been made in this paper to list all the uses of any given type of synthetic rubber. In fact, in most cases, only a few typical uses have been indicated. Since there are modifications of each type of synthetic rubber and since properties can be further modified by compounding, it should be realized that the properties listed are not all-inclusive. Other types of vulcanizable polymers also are known, and many more varieties probably will be made.

It has been estimated that the world's production of synthetic rubber at the end of the war will be 1,200,000 tons per annum (3). This is probably a conservative figure, since it is estimated that the United States and Canadian production will reach a figure of 1,145,000 tons. The Russian and German productions are unknown. What the future of synthetic

rubber will be after the war, as far as volume of production is concerned, is unknown at the present time. However, present trends indicate that synthetic rubber is here to stay and that selected types excel natural rubber for specific uses.

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GERMINATION STUDIES OF SWEET CLOVER SEED

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INTRODUCTION

The failure of viable seeds to absorb moisture and germinate when placed in an environment favorable for germination, a condition of seeds known as "hardness," is very pronounced in sweet clover. Mature ripened seeds while still on the mother plant and for some time after they have fallen to the ground in natural seedings approximate 100 per cent in hardness, and when harvested and hulled by hand to avoid scratching or cracking the seed coats, hardness generally ranges above 90 per cent. Occasional individual plants and some recently isolated strains are comparatively soft seeded, their seeds sometimes running as low as 50 per cent hard, but the species and strains generally grown in both the yellow and white blossomed forms are notably hard seeded; despite the softening effects of the abrasions and other injuries of seed coats by the combine or whatever type of harvester used, hardness still ranges as a rule from 60 to 80 per cent.

The prevalence of hardness accounts for the general practice of scarifying the seed before sowing. Sown without scarification and also in natural seedings, the seeds do not germinate until their coats are made permeable by a period of weathering, usually several months. Most unscarified, hard sweet clover seeds, when sown in the field at the usual time in the spring, do not germinate till the next spring, as has been observed by Hume (10), Kirk (12), Schmidt (14), Whitcomb (20), and others. The weathering through the cool season is much the most effective in opening the seed coats in the field, according to the observations of Harrington (7), Hume (10), Stevens and Long (17), Schmidt (14), and Whitcomb (20, 21).

The object of the investigations herein reported was to obtain more definite information about the particular factors involved in softening the seed coats of sweet clover both in the field and in different types of storage. The investigations cover the conditions to which the seeds are exposed in natural seedings in the field, and the more or less artificial conditions to which the seeds are exposed when stored (1) in constant temperature rooms, (2) in laboratories and dwellings, (3) in unheated open buildings such as granaries, machine sheds, and open garages, and (4) at different depths in the soil. The results of these investigations, as well as being of interest scientifically, are pertinent to arriving at the best methods of storage for sweet clover seed.

PROCEDURE AND RESULTS

Softening Of Seeds In Natural Seedings

Over the period of years from 1927 to 1942, the germination of natural seedings was followed through February, March, and April. Two or more samples each month were collected from the soil of at least six different areas which were well seeded by plants that had matured and dropped their seeds on the areas the previous fall. Some of the areas selected were on highway and railway right-of-ways or waste areas on farms, and some were on cultivated plots of fields where unmolested plants had ripened and dropped their seeds. The seeds were taken from the areas in surface layers of soil from which they were washed out in the laboratory. Tests of samples collected in December and January did not differ significantly in percentage of germination from the tests of the February samples and were not continued after the third year of the investigations. Since the immature soft seeds swell and are soon killed by the fall freezes, the samples collected from the field after the fall freezes consisted almost entirely of hard seeds, and they were not found to vary much in germination in the monthly tests until the middle of March or later.

As shown in Table 1, for February the germination of the seeds collected from the field was consistently very low up to March. The rise in germination started between the twentieth and the end of March in 7 of the years and between the first and twentieth of April in 3 of the years. Much of the germination recorded in April was the result of softening in late March. After the softening period, the increase in germination when the temperature was favorable was surprisingly rapid and general on nearly all of the areas observed. Tests of samples collected not more than a week apart frequently differed 60 to 80 per cent in ger-

TABLE 1

Opening of Seed Coats of Sweet Clover in the Natural Seedings in the Field As Shown by the Percentages of Germination in February, March and April. Data Based on Samples Taken at Six or More Different Locations in the Field. Both Range and Mean of Percentages of Germination Are Recorded

Year	Percentage Germination at Following Dates					
	February		March		April	
	Range	Mean	Range	Mean	Range	Mean
1927–28	4–8	6	18–42	24	52–90	78
1928–29	2–14	8	16–84	72	22–98	92
1929–30	2–6	4	32–52	42	62–96	88
1933–34	4–12	6	12–22	14	89–94	90
1934–35	2–12	8	20–90	86	90–100	95
1936–37	6–10	6	10–35	18	85–98	88
1937–38	4–10	6	8–20	12	75–95	85
1938–39	6–8	7	8–10	10	90–100	95
1939–40	8–13	8	12–32	21	90–100	96
1941–42	4–16	7	4–12	6	60–91	88
Average	4–11	6.6	14–40	30.5	64–90	89

mination. Most of the marked increase in the germination shown in the April column was the result of grand openings of seed coats that started early in April.

During the opening period or soon following, if the weather permitted, there was wholesale germination in the field, followed by thick stands of seedlings.

In the natural seedings the seeds were exposed to all the variable weather conditions, such as drying and wetting, and to variations in temperature ranging from lows of subzero (F) to highs considerably above freezing. Being most in evidence, fluctuations in temperature and moisture are suspected of being the primary factors in softening the seeds. The data in Table 1 suggest that the causal agent or agents must operate over a period of months to effect the softening. That there is a time element involved in the operation of factors that open the seed coats also is indicated in the reports of the field studies by Schmidt (14) and others. Unless hard sweet clover seed is sowed early enough in the season to receive a considerable period of winter weathering, the germination is not satisfactory until the seed has had the weathering of the following winter.

There are various reports on the relation of temperature to the softening of seeds. Helgesen (8) found that hard sweet clover seeds which softened very little in laboratory storage were softened very effectively by outdoor seasonal temperature fluctuations. Gadd (5) obtained more than 90 per cent softening in hard red clover seeds by exposing them to fluctuating low temperatures, running as low as 3°C. Exposure to fluctuating temperatures of 20 to 30°C., according to Whitcomb (22), had no softening effect on either hard red clover or sweet clover seeds. Witte (23) obtained some softening of hard red clover seed under fluctuating temperatures of 20 to 30°C., but much more when alternating temperatures were 10 and 30°C. Harrington (7) reported that alternating temperatures between room temperature and 30°C. had no softening effect on the hard seeds of sweet clover, but alternations of 1 to 30°C.. followed by an incubation in a cool temperature softened 30 per cent of the seeds. On the other hand, Busse (2) froze and thawed hard sweet clover seeds 20 times with no softening effects.

From the previous investigations that have been cited, it appears that alternating low temperatures are the most effective in opening hard clover seeds, but as shown in Table 1 the length of the period of the exposure to alternating temperatures is an important factor and may afford an explanation of the discrepancies in results obtained by different investigators on the softening effects of alternating temperatures.

Softening of Sweet Clover Seeds Stored on an Open Porch and in an Unheated Frame Garage

The storage conditions on the open porch and in the unheated frame garage were comparable to those of the common storage places on farms, such as the barns, cribs, granaries, garages, machine sheds, etc., where the seeds are protected from rain and somewhat protected from the

extreme fluctuations in atmospheric moisture but not much protected against wide outdoor temperature fluctuations.

The seed from which the samples for storage were prepared was hand picked in late summer and early fall of each year from field plots, fields, and uncultivated areas and kept in the laboratory until placed under the experimental conditions. Seeds of both the yellow and white blossomed common species were included, but the difference between the behavior of the seeds of the different species did not warrant a separate recording of data.

In the preparation of the samples the seeds were hand hulled, and immature and abnormal seeds were excluded. The selected seeds, in lots approximating 100 and ranging 95 to 100 per cent in viability, were bagged in small pieces of cheesecloth. One of the duplicate sets of bags was stored dry in wide-mouth bottles closed with cotton. The other set, after being immersed in water a few minutes, was similarly stored with some moist filter paper included in the bottle to help maintain the moisture of the chamber. The bottles accommodating each set were in duplicate, one for storage on the porch and the other in the garage. The results from the two places of storage were similar and were combined in the tabulations.

As the data in the tables (Tables 1 and 2) show, the behavior of the seeds stored on the porch and in the garage was similar to that of the seeds in the natural seedings, the periods and percentages of increased germination corresponding closely. As in the case of the natural seedings, there was not much softening of seeds until March, and between the middle of March and April 20 there was a grand softening period. It is to be

TABLE 2

OPENING OF SEED OATS OF SWEET CLOVER STORED IN CHEESECLOTH BAGS ON AN OPEN PORCH AND IN AN OPEN GARAGE, AS SHOWN BY THE PERCENTAGES OF GERMINATION IN NOVEMBER, WHEN PUT IN STORAGE, AND IN THE FOLLOWING FEBRUARY, MARCH, AND APRIL. THE PERCENTAGES RECORDED ARE THE AVERAGE OF FOUR OR MORE TESTS OF NOT LESS THAN 100 SEEDS EACH

	Percentage Germination						
	November	February		March		April 1–20	
Year		Wet	Dry	Wet	Dry	Wet	Dry
1927–28	8	13	12	89	91		
1928–29	6	4	3	7	6	30	84
1929–30	8	4	2	46	38	74	82
1934–35	12	11	14	16	24	73	92
1935–36	9	4	10	98	98		
1933–34	7	6	8	32	38	34	92
1930–31	13	18	27	28	56	30	84
1936–37	12	28	18	68	74		97
1937–38	8	17	19	17	24	34	72
1941–42	6	14	16	32	44	48	86
Average	9	12	13	43	48	45	86

noted that the data in Table 2 show that the seeds stored dry softened as well as those stored wet and gave a much better germination in April, which in a measure was due to the destructive effects of high water absorption and low temperatures on the seeds that opened prior to the germination tests.

As a factor in softening the seeds, it is obvious that fluctuations in moisture were not significant in the bottles where the seeds were kept constantly wet, and they could have operated only mildly in the dry sets because of the obstruction of moisture diffusion in and out of the bottles by the cotton plugs. The results with seeds stored dry in bottles tightly corked were not significantly different. The evidence was conclusive that moisture fluctuations were of minor, if any, importance in softening the stored seeds. Data from other types of storage, as those from storage in the soil, further support the conclusion that moisture fluctuations are not essential. When the seeds were stored at shallow depths in the soil where they were constantly moist and in an air of rather constant moisture, their softening, as shown in Table 4, proceeded as usual.

That hardness of legume seeds varies with the temperature and moisture of the surrounding air is held by the Hamburg school of Applied Botany and has been shown by Stutz (19), Behrens (1), and others to be true in a number of the commonly cultivated legumes, including vetches, lupines, alfalfa, and the red, white, and alsike clovers. It is probable that hardness in sweet clover seed likewise varies with the atmospheric moisture, but if so the variations are within limits that do not prevent high percentages of softening within the usual range of atmospheric moisture.

Softening of Sweet Clover Seeds Stored in Relatively Uniform Temperatures

That sweet clover seeds soften very slowly when stored in a laboratory or other rooms where the temperature is relatively uniform and kept above freezing has been especially noted by Harrington (7), Helgesen (8), Stevens (16), and Whitcomb (20). Some reports show that sweet clover seed softens so slowly when stored where protected from the wide outdoor fluctuations of atmospheric conditions that its germination may change very little over a period of 10 or more years.

A number of investigators report that hard seeds may soften considerably under constant low temperatures, at least, much more than they do under room or laboratory temperatures. Schaffer (15), Gadd (5), and Kamensky and Bogoljubowa (11) report 24 to 100 per cent softening of hard red clover seed under constant low temperatures. Helgesen (8) obtained considerably more softening of hard sweet clover seeds in a constant low (7°C.) temperature than occurred in a constant moderate temperature. On the other hand, Dunn (4), who stored hard sweet clover seeds for 1 week in −10°C. and then from 1 to 10 months in 5°C., obtained no significant softening.

To obtain more data on the softening effects of constant temperatures, the following course was pursued. Separations of hard seeds approximately 100 per cent viable were obtained from germination samples at

the end of the usual allotted period for germination and were stored in different temperatures, as shown in Table 3, in which 2 years of representative data are given. The seeds, in lots of around 100 and bagged in small pieces of cheesecloth, were stored both wet and dry in the respective temperatures. In room temperature and at 10°C., there was the least softening and the least difference between percentages of softening in the dry and wet conditions. In the low temperatures there was considerable softening. In occasional lots a good germination was obtained, but the average germination was not comparable with that of the seeds stored on the porch or in the open garage, as shown in Table 2.

Tests of seeds that had been stored dry for long periods in the laboratory showed a high percentage of hardness. Some samples stored 10 years averaged 80, and some samples stored 17 years still average 66 per cent in hardness.

In the wet storage under the higher temperatures the percentage germination was somewhat affected by the loss of viability of seeds that softened and died during the interval between tests.

The data in Table 3 show that constant low temperatures are considerably more favorable than the constant warm temperatures for the softening of the seeds but are in accord with the data in Table 2 in emphasizing the fact that fluctuations in temperature are of first importance in the softening process. The data in Table 3 are also in accord with the idea that it is in the realm of freezing that temperature fluctuations operate most effectively in the softening of sweet clover seed coats. (Fig. 1).

Softening Effects of Number and Width of Temperature Fluctuations and of Length of Time of Exposure

A single exposure to a temperature as low as –80°C. was found by Busse (2) to have no softening effect on hard sweet clover seeds, but a single exposure to the temperature of liquid air was decidedly effective. He reports that alternate freezing and thawing at ordinary temperatures as many as 20 times over a period of a few days had no softening effect on hard sweet clover seeds.

Studies scattered over a period of years have been made at the Iowa Station on the softening effects of rapid successive alternations of temperature on hard seeds of sweet clover. Hollowell (9) found that alternating freezing and thawing over a period of a few days of both wet and dry seeds, and when accompanied by alternate wetting and drying, had no softening effect as shown by immediate germination tests.

In more recent studies, samples of known hardness and hard seeds of separations taken from the germination blotters have been exposed to various alternating temperatures over periods ranging up to 20 days without significant softening effects. Sets of samples, kept both dry and wet, and exposed in loosely stoppered bottles and between blotters in petri dishes, in twice daily alternations of temperature from –10° to –20° (F.) to room temperature over a period of 20 days, were not significantly

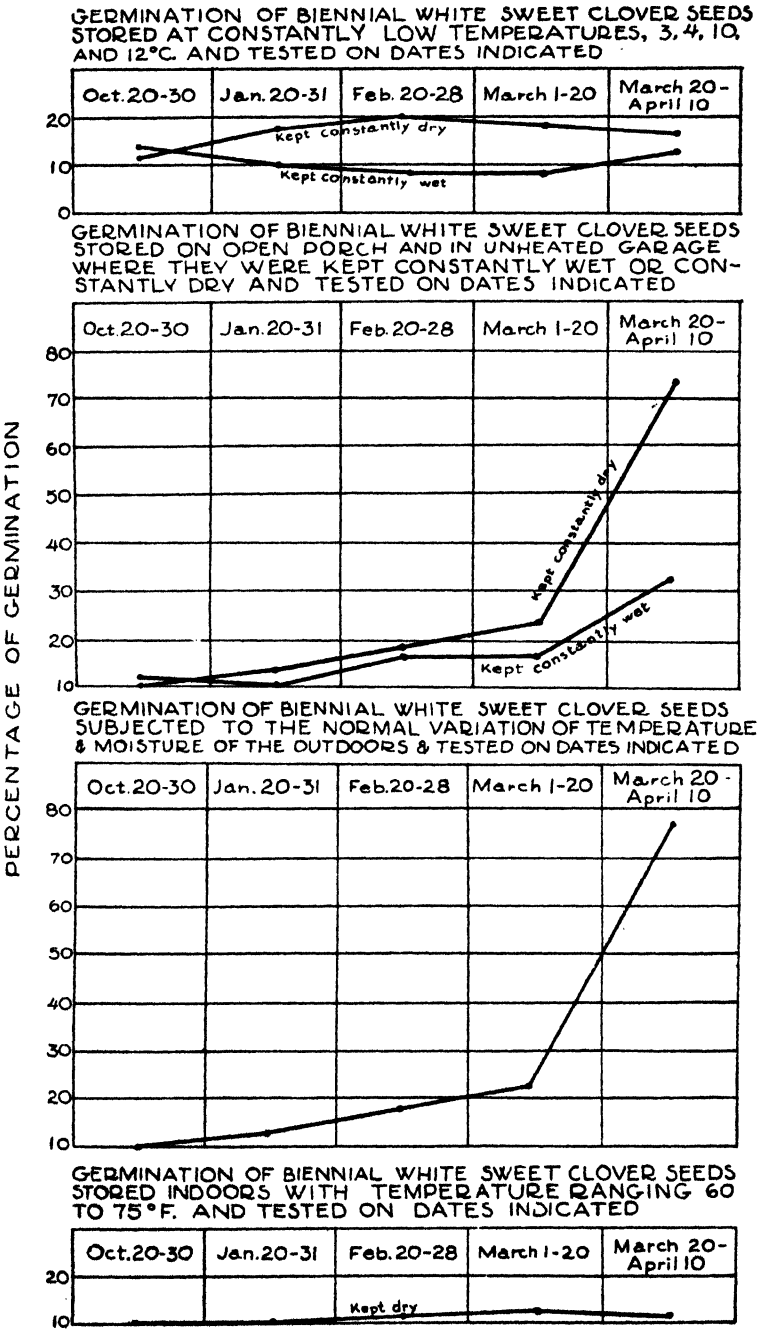


Fig. 1. Graphs showing the germination of sweet clover seeds under different types of storage.

TABLE 3

OPENING OF SEED COATS OF SWEET CLOVER SEEDS STORED UNDER THE CONDITIONS STATED IN THE TABLE. THE DATA ARE THE AVERAGE PER CENT GERMINATIONS BASED UPON FOUR OR MORE TESTS OF EACH LOT OF SEEDS

Date of Tests	Percentage Germination													
	Stored at −3° C.				Stored at 2° C.				Stored at 10° C.				Lab. 15–25°	
	1928–29		1929–30		1928–29		1929–30		1928-29		1929–30		28–29	29–30
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
February	0	3	0	10	3	4	16	20	0	1	8	3	6	2
March	7	1	3	16	3	3	27	20	1	2	4	7	14	3
April	9	2	8	22	4	2	23	22	2	3	6	8	3	5
May	11	0	6	34	3	1	31	16	2	2	5	10	10	4
Average	7	1.5	4	20.5	3	2.5	24	19.5	1.2	2	6	7	8	3.5

softened, although samples similarly prepared and exposed to outdoor temperatures from early February to the middle of March gave a high percentage of germination. Apparentaly neither the number nor the width of the normal temperature fluctuations to which the hard seeds are exposed can compensate for the time required for the softening process.

Softening of Sweet Clover Seeds Buried at Different Depths in the Soil

Records show that sweet clover seed buried well in the soil, can lie dormant many years with very little impairment of vitality. For example, Stoa (18) cites a case in which seed of sweet clover was plowed under. In each of the successive crops to which the field was planted during the following 14 years there was a good volunteer growth of sweet clover, and about 48 hard seeds per peck of soil still were present in the soil the fourteenth year.

In the investigations at the Iowa Station of the softening at the shallower soil depths, the seeds were bagged in pieces of cheesecloth in lots of around 100 and then packed in soil-filled cylinders of screen wire at depths of 1, 3, 6, and 9 inches from the top end of the cylinder. The cylinders were then buried vertically in the soil to a depth flush with the soil surface. Under these conditions the seed remained from sometime in October until late April or after germination in the natural seedings in the fields was well under way.

The cylinders were in duplicate, and one set, after being removed from the soil, was stored in the laboratory and kept moist with the object of checking on the germination that occurred after their removal from the soil to a place of more favorable temperature and aeration. The cylinders of soil, being only about 3 inches in diameter, permitted good aeration and warming of the seeds after their removal to the laboratory. When the cylinders were removed from the soil the seeds at the 1 and 3-inch depths were usually germinating, whereas the deeper buried seeds mostly delayed germination until after the cylinders were removed to the laboratory, where, under the better aeration and more favorable temperature, there was immediate germination. For investigation of seeds buried at deeper depths, the samples in cheesecloth bags were enclosed in loosely closed bottles and small wire screen baskets, and those at the 30-inch depth were provided aeration by means of a gas pipe extending down to their location. The results over a period of years have not been uniform. The summarized data in Table 4 are representative of the usual behavior of the seeds at the different depths.

As the data in the table show, the softening of the seeds varied somewhat inversely with the depth of storage and to a certain extent directly with the fluctuations of temperature at the different soil depths, as a study of the soil temperatures revealed.

At depths of 6 inches or more, no seeds germinated *in situ*. The seeds that germinated immediately after their removal from the soil either did not complete their softening until they were brought to the surface or else, although softened, they were prevented from germinating by un-

TABLE 4

SOFTENING OF SEEDS AT DIFFERENT SOIL DEPTHS AS SHOWN BY THE GERMINATION

Species and Strains	Percentage Germination at Different Soil Depths									
	1 Inch		3 Inches		6 Inches		9 Inches		30 Inches	
	Germ.	Hard	Germ.	Hard	Germ.	Hard	Germ.	Hard	Germ.	Hard
Melilotus officinalis	84	14	62	32	60	32	50	46	8	90
Melilotus alba (biennial)	93	5	46	50	20	79	0	98	12	85
Melilotus alba (Hubam)	92	8	64	33	76	20	10	86	10	87
Average	90		57		52		20		10	

favorable conditions at the lower soil depths. There was also very little absorption of water at the lower soil depths for practically all seeds maintained approximately their dry size until removed to the surface. At the lower depths the germination processes probably are prevented by unfavorable conditions of the soil atmosphere with respect to oxygen and carbon dioxide content.

It is also possible that the imbibing force of the seeds depends much upon chemical changes that are inhibited at the lower soil depths. Chemical changes in purine compounds, such as the breaking down of uric acid and the formation of allantoic acid, have been shown by DeGraeve (3) to occur in the early germinating stages of sweet clover seeds.

Hard sweet clover seeds normally open at the strophiole as shown by Hamly (6) and Martin and Watt (13). It is through the strophiole that the water first enters the seed and contacts the endosperm, which, through its mucilaginous nature and imbibing power, hastens the inward flow of water and its distribution to the distal parts of the seed. It is obvious that the functioning of this mechanism can be much affected by conditions at the lower soil depths.

The seeds at the soil depth of 30 inches, although aerated through gas pipes, remained hard. The explanation is that the temperature conditions at this depth lacked the temperature fluctuations in the realm of freezing which are necessary to soften the seed coats.

SUMMARY

Hard sweet clover seeds, which generally comprise more than 50 per cent of the seed as it comes from the harvester, were investigated as to the weather factors effective in opening their coats to the absorption of water.

Hard sweet clover seeds in natural seedings in the field and when stored over the winter season in unheated open buildings where there was not much protection against temperature fluctuations, were found to soften usually 80 to 100 per cent by the middle of the following April.

Practically all of the opening of seed coats to the absorption of water, however, occurred during the period from about March 20 to April 20 at the Iowa Station.

Hard seeds, wet and dry, opened equally well under the atmospheric fluctuations. The moisture content of the stored seeds did not apparently affect the amount of softening. Dry seeds softened as well under the effect of temperature fluctuations as those stored moist.

Stored under constant temperatures around freezing for several months, hard seeds softened to a maximum of 24 per cent, but when the hard seeds were stored in a constant temperature of 10°C. and in fluctuating temperature, 15–30°C., there was not much softening. The hard seeds, stored over a period of years in the laboratory where the usual temperature fluctuations ranged from 15 to 35°C., softened very little.

It was found that an exposure of 2 months or more to the fluctuations of temperature in the realm of freezing was required to effectively soften the seeds. Attempts to shorten the necessary period of exposure by increasing the frequencies of the temperature fluctuations failed.

When hard seeds were buried in the soil at depths of 1, 3, 6, 9, and 30 inches from October until late in April, the softening varied somewhat inversely with the depth, to the depth of 9 inches. A large percentage of those buried at 1 and 3 inches germinated *in situ*, whereas those that were softened at lower depths did not germinate until they were brought to the surface. Those at the 30-inch depth, where the soil records show that the temperature very seldom drops to freezing, were not changed in hardness.

Evidently fluctuations of temperature in the realm of freezing, acting over a period of 2 or more months, are the factors that normally soften the hard seeds of sweet clover.

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THE PARASITISM OF GLOMERULARIA LONICERAE (PK.) D. AND H. IN LONICERA SPECIES[1]

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INTRODUCTION

The honeysuckle leaf-blight, common and destructive on the foliage of several species of Lonicera, occurs in most of the northeastern states and adjacent provinces of Canada, but reports of its presence, distribution, and the nature of the causal organism have been fragmentary.

Commonly associated with the leaf blight is *Glomerularia lonicerae* (Peck) Dearness and House, and this fungus appears in the literature to be implicated in causing the blight. Recently the presence of a basidiomycete was discovered by James Sinden[3] in the diseased areas of the leaves of Lonicera. This fungus is described and named in this paper. The following study contributed also to the knowledge of the life history of the causal organism, the host-parasite relationship, and the influence of environmental conditions on the response of the pathogen, as well as its geographic distribution and host range.

DISTRIBUTION AND HOST RANGE

The geographical range of *G. lonicerae* roughly comprises the northeastern and north central portions of the United States and adjacent areas of Canada. The states in which the fungus has been found are Massachusetts, New York, Michigan, Wisconsin, Iowa, and the provinces of Canada: Ontario, Quebec, Prince Edward Island, Manitoba, and New Brunswick. *G. lonicerae* also has been reported in Newfoundland. Thirty-three species and varieties (Table 1) of Lonicera have been found to be hosts, 22 of which are new. Infection trials showed that *Symphoricarpos albus* also was susceptible.

DEVELOPMENT OF THE PATHOGEN ON THE HOST

SYMPTOMS AND SIGNS

Some of the first honeysuckle leaves to develop in the springs of 1940 and 1941 exhibited typical leaf blight symptoms. The first symptom

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TABLE 1

HOST RANGE OF *G. lonicerae* ON SPECIES OF LONICERA AND SYMPHORICARPOS

Host*	Literature†	Communications	Investigations by Author	
			Natural Infection‡	Artificial Infection§
Lonicera species	New Brunswick (7, 8, 9)	Wisconsin‖		
amoena Zab.			x	x
bella Zab.			x	x
bella var. *atrorosea* Zab.			x	x
bella var. *candida* Zab.	Ontario (5)		x	x
canadensis Marsh.	Wisconsin (10)	Ontario¶		
	New York (11, 13, 21)	Quebec¶		
	Manitoba (2)			
coerulea L.		Newfoundland¶		
dioica L.				x
discolor Lindl.	Ontario (5)			
gracilipes Miq.				x
korolkowii Stapf.			x	
korolkowii var. *floribunda* Nichols			x	
maackii Max.				x
minutiflora Zab.			x	x
morrowii A. Gray			x	x
morrowii var. *xanthocarpa* Teus.			x	
muendeniensis Rehd.			x	x
nervosa Max.			x	
notha Zab.			x	x
oblongifolia (Gold.) Hook.	Wisconsin (10)			
orientalis Lam.	Ontario (5).			
prolifera (Kirchn.) Rehd.			x	x
prostrata Rehd.			x	x
quinquelocularis Hardw.			x	
ruprechtiana Reg.				x
sempervirens L.				x
tatsienensis Franch.			x	
tatarica L.	Quebec (6, 7, 8)	Michigan**		x
	Prince Edward Island (8)			
	Manitoba (2)			
tatarica var. *alba* Loisel.	Iowa (12)			
tatarica var. *angustifolia* (Wend.) Kirchn.			x	x
tatarica var.. *latifolia* Loud.			x	x
tatarica var. *pallens* Rehd.			x	x
tatarica var. *rosea* Reg.		Iowa††	x	
vilmorinii Rehd.			x	
Symphoricarpos albus Blake				x

* Authorities and spelling after Rehder (Alfred Rehder. Manual of Cultivated Trees and Shrubs. Macmillan Co., N. Y. 1940) unless otherwise noted.

usually observed was a slight yellowing of the diseased portion of the normally green leaf. This change appeared 10 to 18 days after exposure to infection. Occasionally the color change was preceded by a very slight rolling or crinkling which developed 8 or more days after repeatedly exposing very young leaves to basidiospores.

The yellow-green diseased area became tan colored within 3 to 6 days, but the color change was not uniform over the entire area. The veinlets and small portions of the islands bounded by the veinlets became tan or brown sooner than other parts. This difference gave the diseased area a somewhat speckled appearance when viewed with a dissecting microscope and was caused by necrosis of scattered cells usually in the border parenchyma. In contrast to the appearance of the veinlets, the veins retained their green color until the remainder of the diseased area had turned brown (Fig. 1). Advanced stages of the disease appeared as necrotic, dry, brown areas involving either an entire leaf or portions of it. Leaves with large diseased areas usually became rolled and twisted (Fig. 2) and often fell earlier than healthy leaves. The yellow-green borders between the diseased and healthy tissues were very indistinct in the earlier stages of infection but became slightly more definite later.

Signs of the disease were confined to the sporulating stages of the causal agent. Two spore forms occurred: basidiospores (Fig. 3) and conidia (Fig. 4). The former appeared before the latter and always developed on the diseased portion, usually the lower surface of the leaf, under humid and moderate temperature conditions. Appearance of the basidia, as evidenced by a thin, whitish layer, did not occur until the tan stage of the disease. Such basidial-bearing leaves were observed in the spring, summer and fall during and after every period of prolonged precipitation.

The conidial stage, which was less common than the basidial, appeared as a white powdery mass on either surface of the leaf but more frequently on the lower. It developed most abundantly on leaves that were entirely diseased and in a shaded location. Conidia-bearing leaves always could be found during the summer, fall, and early winter on the ground beneath bushes that had borne diseased leaves. In such locations the leaves, which were covered by other leaves and thereby kept moist, bore the most conidia.

HISTOLOGICAL OBSERVATIONS

Methods: To observe host-parasite relationships at intervals after infection, leaves of *Lonicera bella candida* were collected, 3, 8, 12, 15, and 22

† Numbers refer to "Literature Cited."

‡ Observations at the Arnold Arboretum, Cambridge, Massachusetts (August, 1940), Iowa State College Campus, and Horticultural Farm, Ames, Iowa (1940 and 1941).

§ Results of greenhouse and field inoculations, Ames, Iowa (1940 and 1941).

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Fig. 1. Typical symptoms on infected *Lonicera bella candida* leaves.
Fig. 2. Leaves destroyed on *L. bella candida* as a result of a heavy infection of young leaves.

days following exposure to infection; they were killed, sectioned, and stained. A weak, chrome-acetic acid-formalin solution was used for killing, followed by dehydration in dioxan and imbedding in paraffin. A safranin-fast green combination was used to stain general structures; and

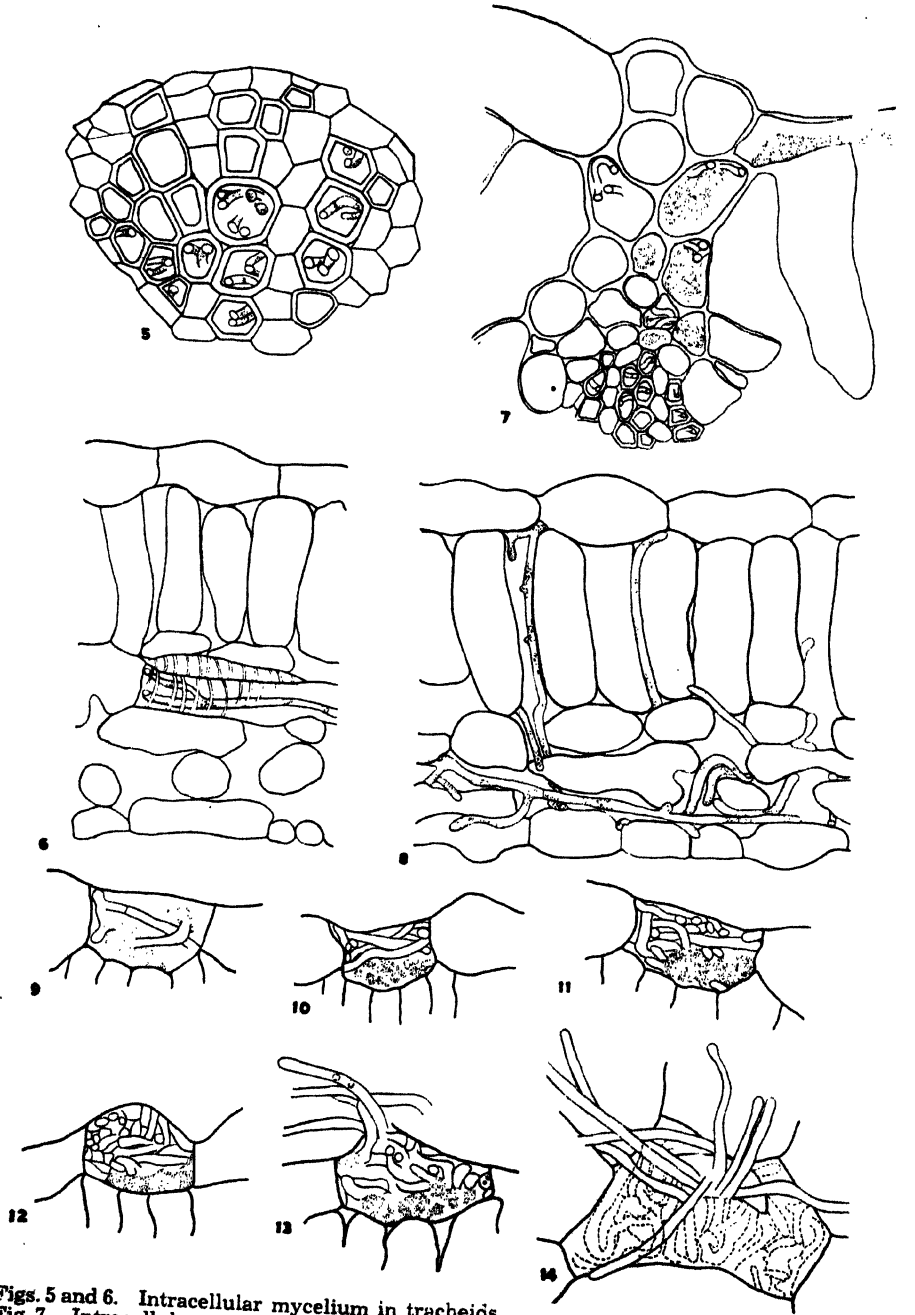


Fig. 3. White layer of basidia on lower surface of infected *Lonicera bella candida* leaves.
Fig. 4. White layer of conidia on lower surface of infected *Lonicera candida* leaves.

iron haematoxylin was used to stain nuclei. Sections were cut from 5 to 18 μ thick. Gross structure was observed in sections mounted in a solution of lactophenol and cotton blue.

Observations: No hyphae were observed prior to the twelfth day, although hundreds of sections from the third and eighth-day collections were examined. On the twelfth day hyphae were abundant in the xylem (Figs. 5 and 6). Intercellular mycelium was not found.

Sections of the fifteenth-day set exhibited abundant intercellular hyphae within the xylem and also some intercellular hyphae in the spongy mesophyll and palisade tissues (Fig. 8). Hyphae also were present within dead upper epidermal cells, border parenchyma, and other mesophyll cells (Figs. 7 and 9-14), which indicated that the hyphae probably pene-



Figs. 5 and 6. Intracellular mycelium in tracheids.
Fig. 7. Intracellular mycelium in mesophyll above vein.
Fig. 8. Intercellular mycelium.
Figs. 9–14 incl. Development of hyphal masses in upper epidermal cells. (9 to 13) from cross section, (14) from surface view of leaf. Stippled masses in host cells represent gum-like deposits.

trated from the xylem through dead border parenchyma cells and thence into intercellular spaces. In the border parenchyma and mesophyll the hyphae never completely filled the cells, as they usually did the upper epidermal cells.

By the twenty-second day both intercellular and intracellular hyphae were very abundant, the latter filling numerous upper epidermal cells. Basidia, arising from hyphae in substomatal cavities, were present on the lower leaf surface.

Both intercellular and intracellular hyphae were septate, hyaline, occasionally branched, and lacked clamp connections. The older portions of such hyphae were practically devoid of protoplasm, whereas the younger growing tips were densely filled. Measurements of 100 hyphae gave a range of 1.4 μ to 3.7 μ and an average of 2.2μ in width.

Some of the intercellular hyphae were congregated in the substomatal cavities, giving rise first to basidia and later to conidia. Each intercellular space between the palisade cells in the diseased area usually was occupied by a hypha by the time the basidia had formed. Some of these hyphae penetrated into upper epidermal cells, where they formed cellular masses.

Host cells were noticeably affected by the presence of the pathogen. In sections collected the twelfth day, scattered dead border-parenchyma and other mesophyll cells were found adjacent to or near veins containing hyphae. Many more cells were dead by the fifteenth day, and, as before, most of these were of the border parenchyma, but a few were mesophyll and epidermal cells. Cells into which hyphae had penetrated, such as those of the upper epidermis, always were dead. By the time conidia had begun to develop, almost all of the lower epidermal cells, as well as many cells in the spongy mesophyll and some in the palisade and upper epidermal layers were collapsed. Very often at this stage all cells in a certain area, such as that above veins, were dead. Sections collected on the twenty-second day contained numerous dead cells. Most lower epidermal and many spongy mesophyll cells were so crushed at this stage that in cross-section they often could not be distinguished from old pieces of hyphae.

Hyphae ordinarily were found in close association with dead cells; sometimes in contact and sometimes within the cells. Some dead cells, however, were observed not in contact with hyphae, and some healthy cells were found in contact with the fungus. As a general rule, though, it appeared that contact of a hypha and cell soon led to the death of the latter.

Dead cells ordinarily were conspicuous by the presence of a dark-staining gum-like mass within them. Such a mass often rendered observation of mycelium within dead cells difficult.

Hyphal masses in honeysuckle leaves generally were found in epidermal cells above veins or veinlets. They usually arose from a binucleate hypha which penetrated into the cell from intercellular binucleate mycelium in the palisade parenchyma. The host cells always were dead at the earliest observed stage of hyphal penetration, and the protoplasm was gum-like and usually massed in the lower portion of the cell. Continued development of the hypha resulted in formation of a compact hyphal

mass completely filling the host cell and somewhat distending the walls. When such a mass was examined from above the surface of the leaf, the hyphae appeared more or less coiled. Most of the cells of such hyphae were filled with protoplasm of moderate density, but the hyphal tips, which were congregated near the upper host wall, contained very dense protoplasm. The hyphal cells were binucleate.

Continued enlargement of the hyphal mass ruptured the upper epidermal cell wall. Several hyphal tips then emerged, becoming slightly constricted as they did so, and grew out over the leaf surface for a distance of about twice the length of the host cell. Under very moist conditions these hyphae developed to 5 or 10 times their normal length and became septate and sometimes branched. Such protruding hyphae never were observed to bear spores or to penetrate adjacent cells.

Similar hyphal masses have been described by Lind (16) and Jackson (14) for *Herpobasidium filicinum* (R.) Lind. Both authors describe them as coiled hyphae, and Jackson noted the binucleate condition of their cells. Lind suggested that they might be reservoirs of nutritive materials, and Jackson referred to them as haustoria. Similar hyphal coils were observed by Lind (17) in *H. struthopteridis* (R.) Lind.

Basidia: The basidial stage was first noticeable on the lower surface of the leaf as a thin, whitish, appressed layer consisting of basidia arising from internal hyphae. This layer became somewhat thicker under continued moist conditions, a phenomenon attributable to the abnormal elongation of basidia and sterigmata and germination of discharged basidiospores lodged on the leaf surface. There was no evidence of a mycelial layer comparable to a rudimentary hymenium.

Basidia arose in the substomatal cavities from hyphae which were few in number at first but later became very abundant (Figs. 15 and 16). A few basidia were observed whose connection with hyphae could definitely be traced and the shape of their basal portion ascertained. Some of the latter were straight and cylindrical in shape (Fig. 18), whereas others were somewhat swollen at the point of contact with guard cells (Fig. 17). Such swellings appeared to be a result of pressure rather than of normal development.

The basidia emerged from stomata as straight cylindrical structures which became curved upon continued growth until they were semicircular in shape and touched, or almost touched, the leaf surface (Figs. 20–24). The diameters of these "semicircles" ranged from 14.0–35.1 μ with an average of 23.4 μ in measurements of 30 basidia; the width of these basidia ranged from 2.3–5.8 μ with an average of 4.4 μ.

Four small protuberances next appeared on the curved basidia at more or less regular intervals and developed into conical sterigmata. Measurements of 45 of these ranged from 7.0 μ–16.4 μ x 1.9 μ–4.7 μ with an average of 10.3 μ x 2.6 μ. Under extremely moist conditions the sterigmata continued to elongate without producing spores. Three or four septa appeared in the basidium soon after initiation of sterigma formation, and the development of spores soon followed. Basidial cells at this stage

were rather highly vacuolated as contrasted to the dense protoplasmic condition in the young basidia. The basidia collapsed soon after spore discharge. Basidia continued forming and emerging from substomatal hyphae until often six or more were present at each stoma. Such a development usually resulted in rupture of the adjacent host cells which already had been killed.

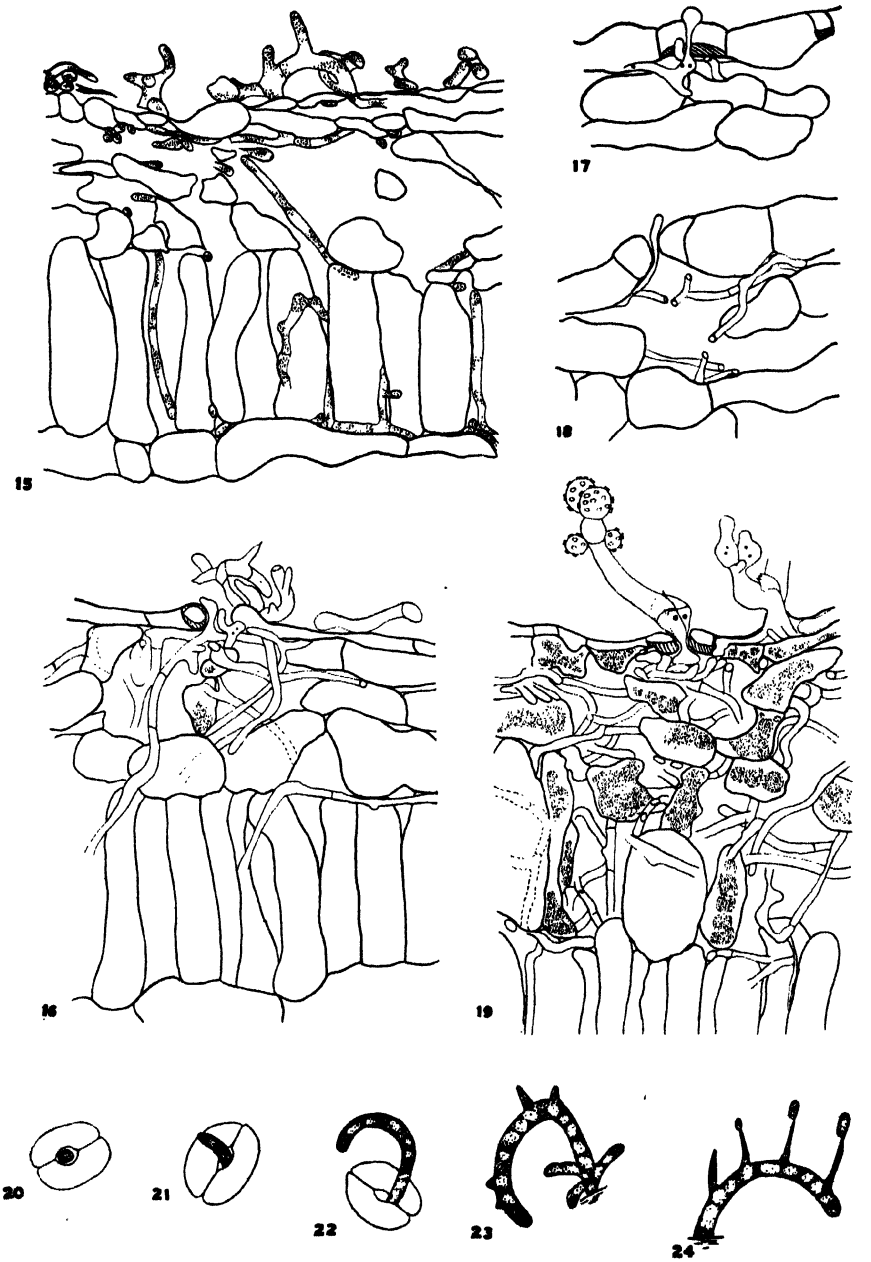
Basidia usually developed on lower leaf surfaces under natural conditions, but when diseased leaves were inverted and placed in a moist chamber the basidia appeared on the upper surface as well. Under such conditions they developed from hyphae within the upper epidermal cells and became intermingled with the hyphae protruding from the cellular hyphal masses.

The nuclear cycle in the basidium was similar to that of many of the basidiomycetes. From the binucleate hyphae below the stomata, a pair of nuclei migrated into the young basidium as it emerged from the stoma (Figs. 25–34). These nuclei fused; the fusion nucleus divided; and the resulting two nuclei migrated to opposite ends of the basidium. This division occurred about the time that sterigmata began to form. These nuclei then divided again. After the second division, crosswalls developed, forming four uninucleate cells. Each nucleus migrated from the basidial cell into the spore when the latter was about half mature, and the nucleus became definitely elongated as it passed through the narrow portion of the sterigma. The nuclei were relatively small in comparison with nuclei of host cells and were often difficult to discern. Distinct chromosomes, which were apparently few in number, were observed only a few times.

Basidiospores: Basidiospores first appeared as small hyaline protuberances at the end of sterigmata and enlarged gradually until they reached mature size. Discharge followed formation of a water drop near the junction of the spore and sterigma. The mature spores were hyaline, uninucleate and cylindrical with rounded ends except for the presence of an apiculus at the attached end (Fig. 35). Only one spore was produced on each sterigma. One hundred basidiospores collected on plain agar and measured immediately ranged from 8.9 μ–12.9 μ x 5.2 μ–7.5 μ, with an average of 10.9 μ x 6.6 μ in size.

Various factors were tested for their influence on basidiospore formation and discharge to determine the exact conditions required for the production of sufficient inoculum for infection trials. The relation between the stage of disease and basidiospore development was tested by the following method. Diseased leaves that did not exhibit basidia were divided into four lots ranging in stages of disease from yellow-green to brownish-black. These leaves (40 to 50) were then affixed with agar to the inner side of the lid of a petri dish, the bottom of which was partially filled with 2 per cent plain agar to maintain a high humidity. The dishes were placed at 20°C. and were examined daily for spore discharge from each leaf, as evidenced by a visible deposit on the agar beneath the leaf.

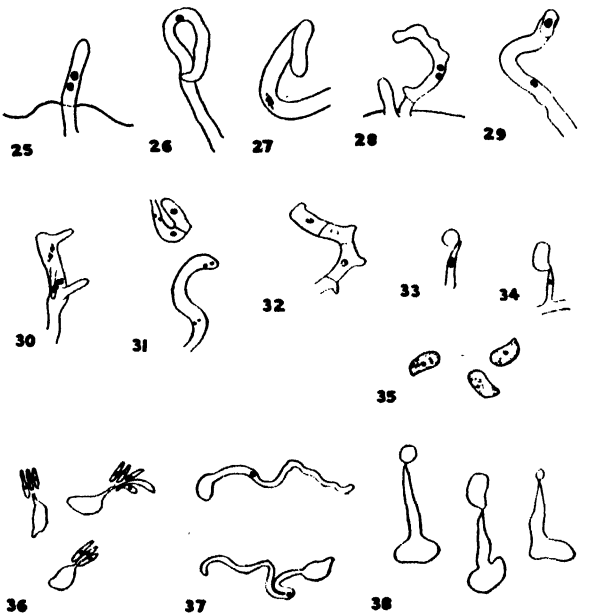
Basidia and basidiospores did not form on leaves until the infection had developed to the point where the tan stage of disease was evident.



Figs. 15 and 16. Extent of mycelium within leaf at time of basidial formation.
Fig. 17. Immature basidium with swollen base.
Fig. 18. Immature basidium with straight base.
Fig. 19. Extent of mycelium within leaf at time of conidial formation.
Figs. 20–24 incl. Stages in development of basidium.

By this time the fungus had reached a stage of development where sporulation occurred within 24 hours when such leaves were placed at 20°C. in a moist environment.

Other factors tested and the general results obtained were as follows: The optimum temperature for basidiospore discharge was between 14° and 21°C.; discharge continued longer at 11°C. (for 8 days) than at 5°, 18°, and 28°C.; more discharge occurred at relative humidities of 89.9



Figs. 25–34 incl. Nuclear cycle during basidial development.
Fig. 35. Basidiospores.
Fig. 36. Uninucleate sporidia.
Fig. 37. Germ tubes from basidiospores.
Fig. 38. Secondary spores from basidiospores.

per cent and 100 per cent than at lower ones; and the number of basidiospores discharged from an infected leaf section (9 x 19 mm.) was estimated at 974,700 over the 91 hours.

Conidia: Conidial development followed basidial formation on the same area or at the margin of it. Conidia sometimes began forming before basidial production had ceased, and under such conditions the conidiophores became intermingled with the basidia. When abundant, the conidia formed a white powdery layer.

Conidiophores developed from binucleate hyphae of the same general size and shape as those from which basidia had previously formed (Fig. 19). The young binucleate conidiophores were of the same general shape at emergence through the stomata as were basidia and possessed basal portions which were sometimes uniform in size and sometimes slightly swollen

at the point of contact with guard cells. Only one conidiophore usually emerged from a stoma at first, but this one was soon followed by several others until three or more were present in a clump.

The young condiophores were binucleate, cylindrical, and densely filled with protoplasm (Figs. 39–47). As they developed, they soon became swollen to about twice their original size, and their terminal portion became separated from the basal part, first by a slight constriction, later by a septum. A binucleate protuberance next appeared just below the septum, enlarged to a size approximating that of the terminal portion, and was cut off by a cross wall from the stalk cell. From each of the two apices, two other binucleate protuberances developed in succession. Each of the original two cells became transformed into stalks, and the four terminal cells developed into spherical binucleate conidia. While the latter were still immature, two additional binucleate protuberances began to form, one on either side and below the junction of the two stalk cells. Such structures enlarged and became transformed into sessile, conidia-like bodies. Instead of being spherical like the others, however, they were elongated. Beginning with the oldest spores, which were the inner and the smallest ones, all conidia became verrucose as they reached maturity. When the conidia dropped off they usually did so as a clump rather than individually. The thin-walled upper portion of the conidiophore then collapsed, but the swollen, thick-walled basal portion remained erect.

Measurements of conidia and conidiophores are recorded in Table 2. Measurements of length and width are given for the elongated conidia,

TABLE 2

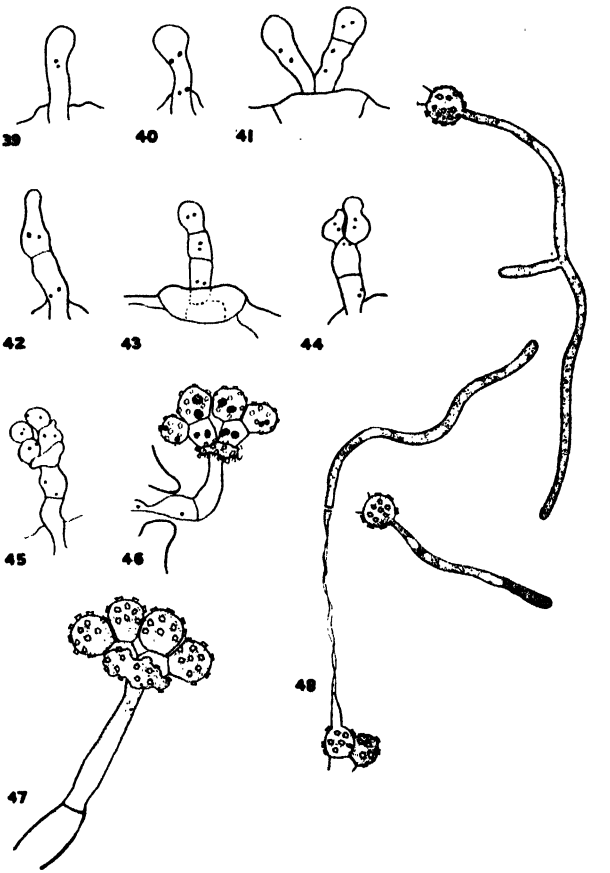
MEASUREMENTS OF CONIDIA AND CONIDIOPHORES

Structure	Size in Microns	
	Range	Average
Type of Conidia		
Outer	10.2–17.0	12.6
Inner	8.5–13.6	11.3
Elongated	5.1–13.6×11.9–22.1	9.3×17.0
Conidiophore		
Width of upper portion	2.7–5.1	3.7
Width of lower portion	5.8–8.5	7.1
Total length	30.6–54.4	40.8

but only the diameter is given for the other conidia, since they were nearly spherical. The three types of spores differed considerably in size. Of particular interest is the fact that the average diameter of the inner conidia was 1.3 μ less than that of the outer conidia. Other differences between these two types of spores have been observed and will be discussed in later paragraphs.

A short time before the terminal cell was cut off from the remainder

of the young conidiophore, a septum developed about midway between it and the conidiophore base. Another enlargement usually formed just beneath this septum and developed into a conidiophore and conidial mass similar to that of the original stalk. The protuberance received its nuclei by a pair migrating from mycelium within the leaf. All later stages were binucleate. This second group was about half mature by the time the first



Figs. 39–47 incl. Stages in the development of conidia showing nuclear condition.
Fig. 48. Conidial germination.

group was completely developed. Occasionally more than one branch conidiophore developed from the original stalk, but this was unusual.

A definite difference of spore reaction was noticed with each of the following stains: iron-haematoxylin, gentian violet, fast green, and cotton blue. The outer two spores in each clump were stained darker, and their contents appeared more homogenous than those of the other spores. With the iron-haematoxylin stain the nuclei of the outer spores appeared very dense, whereas those of the inner spores and stalk cells were larger and

less dense (Figs. 39–47). These reactions were interesting, since only rarely did any spore other than the outer type germinate when the conidia were placed under various conditions of temperature, light, etc.

The conidia were definitely binucleate, but different methods usually were necessary to distinguish the two nuclei in the different spores. Nuclei in the inner and elongated conidia were stained best when the spores were dusted on agar on a slide, partially dried, killed, and stained. Nuclei of the outer conidia were demonstrated best in leaf sections cut in paraffin and stained.

Experiments to determine the effect of various factors on conidial formation showed that changes of temperature and the stage of the disease influenced conidial formation more consistently than any of the other factors studied. The effect of the stage of the disease was determined by collecting nonconidial-bearing, diseased leaves and dividing them into two lots, one exhibiting the yellow-green to tan color stages of disease and the other the brownish-black stage. These lots were wrapped separately in moist cheesecloth and placed in an incubator at 15°C. After 5 days conidia were present on only 2.5 per cent of the 120 leaves exhibiting the early stages of disease, whereas 20.0 per cent of the 105 leaves exhibiting the later stages, bore conidia. This tendency for conidia to develop most abundantly on older leaves has been observed in other experiments and in the field. Additional studies indicated that conidial formation was more abundant at intermediate (8, 11, 13, and 18°C.) than at extreme (5, 23, 25, and 31°C.) temperatures.

CHARACTERISTICS OF THE PATHOGEN IN PURE CULTURE

A study was made of the pathogen in pure culture. Preliminary trials had demonstrated that the organism grew freely, although slowly, on artificial media. Additional experiments were made to determine the rate of growth, longevity, and sporulation of mycelium in culture.

Mycelium was hyaline, septate, occasionally branched in culture, and lacked clamp connections. Hyphal fusions never were observed. Hyphal width ranged from 1.4 μ–3.7 μ and averaged 2.2 μ for 208 measurements. This is the same average width as that found for hyphae in diseased leaves. Aerial mycelium appeared white on agar and submerged mycelium a very light tan. Two distinct areas of surface growth normally were present, a thin peripheral and a thick central growth, the latter developing at the point of inoculation.

Usually the aerial mycelium made a uniform, rather loose, pubescent growth, but sometimes it became matted or tufted. The characteristic type of growth, with slight variations, appeared on potato-dextrose agar, carrot-decoction agar, oatmeal agar, cornmeal agar, malt agar, and Lonicera leaf decoction agar, but on sterilized Lonicera leaves the growth was thin and villous.

Mycelial growth was exceedingly slow in culture. The rate of growth of mycelia arising from single basidiospores, from masses of basidiospores, and from clumps of conidia was determined by the following experiment.

Spores, or spore masses, were transferred on agar blocks of the same size to carrot-decoction agar slants. After 29 to 40 days the rate of growth was determined by measuring the length of mycelial growth in the tubes. The average daily growth from single basidiospores was 0.14 mm., from conidial clumps, 0.28 mm., and from masses of basidiospores, 0.41 mm.

LONGEVITY OF MYCELIUM IN CULTURE

Some difficulty was experienced in keeping the organism viable in culture. The two factors which were assumed to have the greatest effect on such viability were temperature and drying of the substratum. The effects of both were tested in the following experiment. Masses of basidiospores on approximately equal portions of carrot-decoction agar were transferred to separate carrot-decoction agar slants. Eight days later, when all transfers exhibited mycelial growth, four tubes were placed in each incubator, held at the following temperatures: 10, 18, 26, and 33°C. Four more slants also were placed at 18°C. in a desiccator containing calcium chloride in order to test the effect of drying on viability of the mycelium. Isolates were made at intervals from each of these cultures by transferring portions of the mycelium to carrot-decoction agar slants.

Mycelium died much sooner at high than at low temperatures. It was no longer viable at 33°C. at the end of 13 days but was still viable at 10°C. after 147 days when the experiment was discontinued. The time of mycelial death was intermediate at 18 and 26°C. Cultures that were dried rapidly at 18°C. lost their viability much sooner than cultures at the same temperature allowed to dry more slowly under normal conditions.

One of the primary objectives of the cultural studies was to determine whether one spore form could arise from a plating of the other on artificial media. This objective was partially achieved in preliminary experiments when conidia developed in cultures that had originated from a mass of basidiospores. Such results made it desirable to determine whether conidia would arise from single basidiospore isolates.

Several platings were made of single basidiospores and, for comparative purposes, of masses of basidiospores and of single clumps of conidia. Pairings were made later of different combinations of mycelia from some of the single basidiospore isolates. All platings were made from May to July, 1941, and examined at intervals until February 2, 1942, when a final examination was made.

Basidiospores did not form in any of the cultures, whereas conidia developed from all types of isolates. Conidial formation occurred in the following percentage of trials from the various types of isolates (Table 3): 66 per cent of massed basidiospores, 68 per cent of single basidiospores, 70 per cent of paired mycelia, and 79 per cent of conidia isolates. This tendency of conidial isolates to develop conidia more frequently than other types of isolates also had been observed in previous cultural trials. Single basidiospores generally gave rise to conidia later and masses of basidiospores earlier (about 3 to 6 weeks after plating) than did the conidial and paired mycelial isolates. Many isolates of all types failed to develop

TABLE 3

PRODUCTION OF CONIDIA IN CULTURE BY VARIOUS TYPES OF ISOLATES

No. of Tests	Spore Forms and Mycelia Plated on Agar	Number of Cultures Exhibiting None, Few, Several, and Many Conidia				Total No. of Cultures
		None	Few	Several	Many	
9	Single basidiospores	12	19	6	1	38
2	Mass of basidiospores	11	14	7	0	32
1	Single clumps of conidia	3	9	2	0	14
1	Paired cultures of mycelia from single basidiospore isolates	6	8	3	3	20

conidia in these experiments and in other cultural trials. This behavior may be associated with the same factor causing the erratic appearance of conidia on naturally infected bushes.

The growth of mycelia from single basidiospores was relatively slower and that from masses of basidiospores relatively faster than that from other types of isolates.

NAME OF PATHOGEN

The name *Glomerularia lonicerae* (Pk.) D. & H. has been applied to the pathogen up to this point for the sake of a logical elucidation of its historical and structural characteristics. The discovery of a basidium, however, necessitates the use of a name based upon this structure, since it represents the perfect stage.

The basidial stage is characterized by curved, four-spored, transversely septate basidia which emerge through stomata from hyphae within honeysuckle leaves; do not form hymenia; and develop intracellular masses of mycelium in addition to intercellular mycelium. These characteristics place it in the genus Herpobasidium, one of the Auriculariaceae.

The genus Herpobasidium was composed of two species, both parasitic on ferns. It was erected by Lind (16) in 1908 to accommodate a fungus previously described independently as *Gloeosporium filicinum* by Rostrup (22) and as *Exobasidium brevieri* by Boudier (3). Instead of presenting a formal description of the new genus and species, Lind gave merely a general discussion. The most pertinent facts contained in this discussion are as follows:

The fungus begins to appear on fern leaves as soon as they reach their mature size, apparently in May or June. Hyphae, which have formed in the substomatal cavities, emerge through stomata, creep along the epidermis and form a nongelatinous subiculum upon which basidia are produced. These areas appear as small white flakes on the under side of the leaf and are about 1 mm. thick, 2 mm. wide, and 4 mm. long. The basidia are two-celled and measure 9 μ x 40–50 μ. They develop two sterigmata and two spores, the latter of which are pear-shaped, hyaline, unicellular and measure 5–8 μ x 10–18 μ in size. Mycelium within the leaf is both intercellular and intracellular. The latter, which occurs in scattered cells,

develops about the same time as does the subiculum. Such intracellular mycelium is coiled and at first hyaline, later brownish. The organism apparently over-winters as perennial mycelium in rhizomes.

Jackson (14) also studied this species and found a prevailing binucleate condition prior to basidial formation. The two nuclei fuse in the basidium; the fusion nucleus divides once; and each of the two spores receives a nucleus. He was unable to germinate the basidiospores in preliminary experiments.

Lind (17) later renamed a parasite on *Struthopteris germanica* Willd. as *Herpobasidium struthopteridis* (Rostrup) Lind but failed to accompany it with an adequate description. This parasite resembles *H. filicinum* in possessing intracellular coils of mycelia and in being apparently perennial but differed by causing deformations of the host.

The Lonicera pathogen differs from both of the known species of Herpobasidium. It resembles *H. filicinum* by being a leaf parasite, developing intracellular masses of mycelium, producing basidia on nongelatinous hyphae and causing indirect cortical necrosis. It differs by not possessing an exterior subiculum, having four-celled basidia and developing masses of mycelia instead of coiled hyphae within the host cells.

The meager description by Lind of *H. struthopteridis* makes a comparison of it with the Lonicera pathogen rather difficult. Lind merely pointed out that the pathogen was parasitic on ferns, caused them to become deformed, and produced the characteristic coils of mycelium within host cells. Otherwise it was apparently similar to *H. filicinum*. The honeysuckle pathogen appears to be distinct from this species. Therefore, since it differs from both *H. filicinum* and *H. struthopteridis*, it is believed to be a new species of the genus Herpobasidium.

The specific name selected for the pathogen is *deformans* to designate the rolling and twisting of leaves which are attacked. The complete name for it then becomes *H. deformans* and is so described.

Herpobasidium deformans sp. nov.

Syn. *Herpobasidium foliodistortum* Gould, nomen nudum. Rept. Iowa Agr. Exp. Sta. 1942-43 (I) p. 136. Rev. Appl. Mycol. 23:331. 1944.

Maculis subcircularibus, subinda confluentibus, foliique paginam fere totam occupantibus, brunneis; basidiis hypophyllis, arcuatis, 3-septatis, 2.3–5.8 μ x 14.0–35.1 μ; sterigmatibus conicis, 1.9–4.7 x 7.0–16.4 μ; sporis hyalinis, cylindricis, 5.2–7.5 x 8.9–12.9 μ; conidiis globosis aut elongatis, asperulis, hyalinis, in binis tres: interioribus, 8.5–13.6 μ, exterioribus 10.2–17.0 μ, lateralis 5.1–13.6 x 11.9–22.1 μ, caducis in glomerulos.

Hab. in foliis vivis *Lonicerae bellae candidae* in America boreale.

Herpobasidium deformans sp. nov.

Syn. *Herpobasidium foliodistortum* Gould, nomen nudum. Rept. Iowa Agr. Exp. Sta. 1942-43 (I), p. 136. Rev. Appl. Mycol. 23:331. 1944.

Basidia forming a thin effused whitish layer on lower leaf surface, emerging through stomata; at first straight, later becoming recurved, and four-celled by formation of transverse septa 2.3–5.8 μ x 14.0–35.1 μ; sterigmata conical 1.9–4.7 μ x 7.0–16.4 μ; basidiospores uninucleate, hyaline, cylindrical, 5.2–7.5 μ x 8.9–12.9 μ, often germinating by repetition, by forming secondary spores, or directly by germ tubes, capable of infecting honeysuckle leaves; conidia often developing later in or adjacent to areas occupied by basidia, producing in mass a white powdery layer, developing from branched conidiophores; latter emerging through stomata, becoming 5.8–8.5 μ wide at base, 2.7–5.1 μ wide at top and 30.6–54.4 μ long, basal portion thick-walled at maturity, upper portion thin-walled and collapsing; conidiophore giving rise to six hyaline, verrucose, binucleate spores, four of these spherical and formed in pairs on stalks, the two outer ones measuring 10.2–17.0 μ and the two inner ones 8.5–13.6 μ; the other two spores forming at junction of the two stalks, elongated, 5.1–13.6 μ x 11.9–22.1 μ; spores dropping from conidiophore as a clump; only outer two spores capable of germination, this by germ tubes; conidia apparently unable to infect honeysuckles. Ames, Iowa. Nov., 1942, on *Lonicera bella candida*. Iowa State College Herbarium. Type.

Conidial stage reported from Massachusetts, New York, Michigan, Wisconsin, Iowa, Ontario, Quebec, Prince Edward Island, Manitoba, New Brunswick, and Newfoundland.

It should be pointed out that the name, *Glomerularia lonicerae* (Pk.) D. & H., is a nomen nudum. This name was listed but the plant never described as a new species by Dearness and House (11) in 1921. They gave as a synonym for it *G. corni* var. *lonicerae* Peck, but Peck (20, 21) never published such a name, although he did record collections of *G. corni* on Lonicera species.

GERMINATION OF BASIDIOSPORES AND CONIDIA

To contribute to our understanding of the life cycle of the pathogen, experiments were conducted to determine the type of germination of basidiospores and conidia. The influence of environmental factors on spore germination was tested to determine the optimum conditions for infection. In addition, the longevity of spores was determined in order to predict the length of time that inoculum could remain viable.

Basidiospores and conidia differed considerably in their methods of germination on agar. The latter germinated only by germ tubes, whereas two types of germination were characteristic of basidiospores. Some of the latter spores formed uninucleate germ tubes (Fig. 37), and others produced uninucleate secondary spores at the end of slender hyphae (Fig. 36). The formation of each of these germination types often was retarded a step by the development of another spore that was similar in shape but slightly smaller than a basidiospore. This spore was produced on the end of a sterigma-like outgrowth from a basidiospore and germi-

nated either *in situ* or after detachment to produce either secondary spores or germ tubes (Fig. 38). Normal germ tubes that had developed from basidiospores occasionally produced the sterigma-like structures and spores. The shortest period found for basidiospore germination was 1¾ hours. Germination usually occurred at the end or side adjacent to the apiculus. Basidiospores that produced secondary spores usually germinated before those that developed germ tubes. Both types of germination occurred on water and carrot-decoction agars, but the type with secondary spores was more abundant on the former, and the germ tube type was more common on the latter. Both types developed into similar mycelia.

The conidia were unique in that usually the two outer spores were the only ones that germinated, although the elongated stalk spore occasionally developed germ tubes. The germ tubes were branched occasionally and binucleate (Fig. 48). Two hours was the shortest time observed for conidia to germinate.

During preliminary investigations conidia were found to germinate only by means of a germ tube. Since they were binucleate and thick-walled, it appeared possible that they might function as resting bodies and under certain conditions, not heretofore discovered, give rise to basidia and basidiospores. Tests were made, therefore, of the germination of conidia under the following conditions, but the method of germination was unchanged: (1) in artificial light, sunlight, and alternately in sunlight and darkness; (2) on water agar, carrot-decoction agar, and honeysuckle leaf-decoction agar; (3) after being frozen, warmed, and alternately frozen and warmed; (4) on thin layers of water agar which provided moisture for only a short time; (5) on water agars made up with different amounts of agar-agar to provide substrata with different degrees of readily available moisture.

The effect of temperature on basidiospore germination was determined in 11 experiments using temperatures ranging from −5 to +33°C. with 2 per cent water agar as the substratum. Best results were obtained by counting the percentage of spores germinating within 6 hours after beginning the experiment; otherwise the production of secondary spores produced erratic results. The limits for germination were found to be near 1 and 33°C. and the optimum between 18 and 26°C (Fig. 49).

In another experiment the effect of storage of basidiospores at extreme temperatures (+35 and −28°C.) was tested. Basidiospores were thin-walled and appeared rather delicate. It was interesting to find, therefore, that some of them were still capable of germination after an exposure of 30 hours to temperatures of +35 and −28°C. Their viability decreased much more rapidly under exposure to high than to low temperatures, "breaking" very rapidly after 22 hours at 35°C. and not until after 30 hours at −28°C.

Preliminary experiments indicated that the minimum relative humidity necessary for spore germination was 97.5 per cent or above. Five

additional experiments demonstrated that the range was from 97.5 to 100 per cent, but very little germination took place below 99.1 per cent.

In conjunction with the study of factors affecting basidiospore germination, certain of these factors also were tested for their effect on conidial germination. The temperature effect first was determined in terms of percentage of spores germinating. Results showed that the minimum point at which germination took place was below 2°C., and the maximum point lay between 33 and 40°C. The optimum temperature, however, could not be found accurately by this method, because the percentage of spores germinating was consistently low. To determine this temperature an indirect method was therefore used, that of measuring the lengths of germ tubes developing at different temperatures on water agar. It should be emphasized that this method involves the effect of temperature, both upon germination and germ tube growth. In the three experiments run by this method the optimum temperature for germination ranged from 22 to 25°C.

In studying the effect of storage at various temperatures, results of two of the experiments showed that conidia retained their viability longest at low temperatures. They rapidly lost their ability to germinate when stored at 15 and 25°C. for longer than 14–15 days, but at 7.5 and —7°C. they were still viable after 40–41 days. In the third experiment conidial-bearing leaves of *Lonicera morrowii* and *L. bella* were stored in an incubator at 7°C. in the laboratory at 20–25°C., and outside on the ground beginning in November. Conidial viability dropped to zero sometime between 96 and 132 days of storage under all conditions in this experiment.

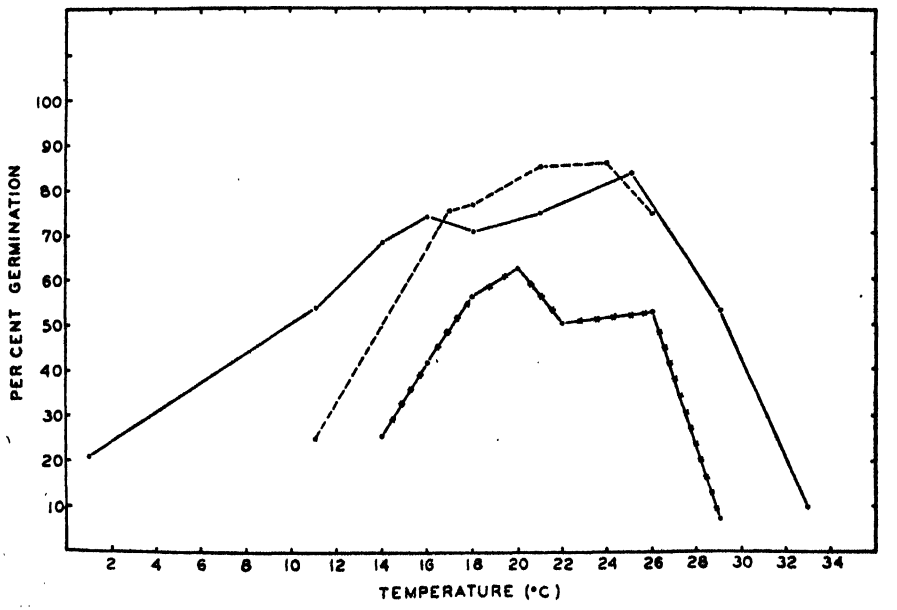


Fig. 49. Influence of temperature on basidiospore germination—3 trials.

After 30 days the percent of germination was lower for the conidia in the laboratory than for those under the other conditions. However, even under cool conditions such spores apparently do not retain their viability long enough to survive the cold winter months of Iowa.

An interesting contrast between conidia and basidiospores is that of retention of viability under storage conditions. The longevity of the latter was measurable in hours but that of the former in days. Both were viable for the longest time at cool temperatures.

While studying the influence of light on the type of conidial germination, data also were obtained regarding its influence on the rate of germination. In three cases out of four the germ tubes were shorter when illuminated than when not, at the same temperature. Light seemed to have more inhibiting effect on germ tube development at 19, 26, and 28°C. than at 22°C., which is nearer the optimum for germination. The germ tubes developing in the light at 28°C. were spherical rather than cylindrical.

INFECTION EXPERIMENTS

The phenomenon of infection was studied using the different spore stages under different environmental conditions. All attempts to secure infection with mycelium and conidia failed. Mycelium was applied to healthy and wounded leaves, buds and stems, but in no case did infection occur. Similar results were obtained when conidia were tried, either fresh or aged, in the greenhouse or in incubators, on leaves or buds. These results were in marked contrast to those obtained with basidiospores, where infection occurred very readily.

BASIDIOSPORES

Temperature: The ability of basidiospores to infect honeysuckle leaves was discovered in preliminary experiments. Then arose the next problem, determining the influence of the most important environmental factors governing infection. During the tests of these factors the general procedure used to expose leaves to infection was as follows: spores from discharging basidia on leaves were collected on plain agar, washed off with distilled water, and sprayed on healthy young leaves of potted plants. These exposed plants were placed in moist chambers for 2 or 3 days and then removed to the drier environment of either a greenhouse bench or an incubation chamber. The number of diseased leaves was recorded 19 to 32 days after exposure. Young leaves were obtained for each experiment by removing all old leaves from plants 10 to 14 days previous to exposure. The following temperature tests were made, using this general procedure, and placing the sprayed plants in moist chambers at different temperatures for 2 or 3 days before transfer to the greenhouse.

The results of seven experiments run in the greenhouse during the winter and spring months of 1941 are summarized in Table 4. From 5 to 46 plants were used in each test at each temperature. The check plants, which were placed in moist chambers in the greenhouse, had a total of

TABLE 4

PERCENTAGE OF *Lonicera bella candida* LEAVES BECOMING DISEASED AFTER EXPOSURE TO INFECTION AT DIFFERENT TEMPERATURES

Exposure No.	1° C.		7°–14° C.		15°–18° C.		19°–21° C.		24°–28° C.	
	No. Leaves Exposed to Infection	Percentage Leaves Infected	No. Leaves Exposed to Infection	Percentage Leaves Infected	No. Leaves Exposed to Infection	Percentage Leaves Infected	No. Leaves Exposed to Infection	Percentage Leaves Infected	No. Leaves Exposed to Infection	Percentage Leaves Infected
1			158	4.4	118	45.8			398	3.3
2			270	16.7	171	53.2			634	5.4
3	185	1.6	314	6.4	246	18.7			202	1.0
4			410	4.4	106	5.7	104	7.7	98	0.0
5			334	13.1	120	23.3	112	13.4	134	0.0
6			472	1.7	440	14.1	472	8.5	334	2.1
7*			1,330	16.4	689	23.2			634	11.2

* Diseased leaves with discharging basidia were suspended in a moist chamber above the plants exposed to infection.

521 leaves, of which none became diseased. The optimum temperature for infection ranged from 15 to 18°C. in these experiments. The percentage of leaves that became infected decreased rapidly at temperatures either higher or lower than the optimum. For instance, within the range 15 to 18°C., more than 14 per cent of the leaves became infected in six out of seven experiments, whereas at other temperatures this percentage was exceeded only twice. The minimum and maximum temperatures for infection appeared to be near 1°C., and 24 to 28°C., respectively. Usually only one lesion appeared on each leaf exposed to infection at temperatures below or above optimum, whereas at the optimum (15 to 18°C.) several lesions ordinarily were present.

Humidity: Plants were exposed to infection during May and June of 1941 by suspending leaves bearing discharging basidia over seedlings in 3-inch pots in a moist chamber for 2 hours. A layer of paraffin and vaseline mixture was next poured over the surface of the soil in the pots, which were then inverted over Mason quart jars with the inverted plants projecting inside. The paraffin mixture formed a seal against diffusion of water vapor into or from the containers. Relative humidities in the jars were regulated by sulfuric acid solutions. Large intervals of relative humidity were used to allow for the effect of transpiration on the increase of relative humidity in each jar. The exposed plants were left in these humidity chambers at 10 and 15°C. for 2 days in the first experiment and for 3 days in the second, then removed to a greenhouse bench and examined after 18 to 21 days for number of diseased leaves. The number of plants used at each relative humidity varied from two to five. None of the 90 check leaves became infected.

The results of these two experiments (Table 5) show that most infection occurred at a relative humidity of 100 per cent, although some took place as low as 70.4 per cent. Infection at the latter relative humidity was surprising, inasmuch as a relative humidity of more than 99 per cent had been demonstrated to be necessary for more than a 2 per cent spore germination. This discrepancy may have been caused by condensation of transpiration water vapor on spores located near stomata.

The length of time necessary for leaves exposed to infection to remain

TABLE 5

PERCENTAGE OF LEAVES INFECTED AT DIFFERENT RELATIVE HUMIDITIES

Exp. No.	Leaves Tested	Relative Humidity—Percentage					
		49.9	60.7	70.4	80.5	89.9	100.0
1	Number exposed to infection	74	70	80	86	100	50
	Percentage infected	0.0	0.0	7.5	0.0	2.0	38.0
2	Number exposed to infection	62	80	60	70	60	90
	Percentage infected	0.0	0.0	15.0	17.0	0.0	50.0

in a saturated atmosphere before infection could occur was tested by placing such plants in a moist chamber and removing them at intervals to a greenhouse bench. These experiments were made at different times from January to July in 1941 with one to four plants for each test. Examinations were made of the number of diseased leaves 20 to 33 days after exposure to infection. In no case did any of the 745 check leaves become infected.

Some infection occurred within 12 hours (Table 6), but the number of diseased leaves was increased considerably by a more extended exposure to a high relative humidity. This statement is best substantiated by the data in Experiment 5, since in the other experiments the air was rather humid in the greenhouse, and some infection probably occurred after the exposed plants were removed from the moist chamber to the greenhouse bench. The greenhouse air was very dry on the day when the plants were removed from the moist chamber in Experiment 5, and subsequent penetration probably was negligible. An exposure of 3 days was necessary to obtain infection of more than 50 per cent of the leaves in this latter experiment, although 20 per cent were infected after only 1 day's exposure. These results indicate that an extended period of damp weather is more conducive to abundant infection than is a short period such as 12 hours.

Leaf age: The results of preliminary infection experiments were somewhat erratic. It was soon discovered that there was a difference in the reaction of mature and young leaves to exposure of infection. In order to facilitate other investigations this reaction of leaves was subjected to test in 1941. Leaves of different ages were exposed to infection according to the method described earlier. The numbers of diseased leaves were recorded 30, 20, and 22 days after exposure, respectively, for the January, March, and June trials described below.

The leaves became resistant as they matured (Table 7), although the age at which this resistance was reached varied in the different experiments. This age was 18, 20, and 12 days, respectively, for the January, March, and June exposure. In none of the experiments did infection occur on leaves 20 days old or older. A greater percentage of the younger than the older leaves within the susceptible age group became infected in all experiments. Some of the younger leaves also had more than one lesion per leaf, but this was true of only a very few of the older leaves.

Leaf surfaces: As a continuation of the studies on relation of leaf condition to infection, three experiments were run to determine whether penetration could occur through both the upper and lower leaf surfaces. These two surfaces had been found to differ morphologically in that the upper surface possessed a relatively thick cuticle and lacked stomata, whereas the lower surface had a thin cuticle and many stomata.

The leaves were exposed to infection in Experiments 1 and 2 by spraying a spore suspension on one leaf surface while the other surface was covered, and in Experiment 3 by brushing a spore suspension on the

TABLE 6

PERCENTAGE OF DISEASED LEAVES DEVELOPING ON PLANTS EXPOSED TO INFECTION AND PLACED UNDER HIGH HUMIDITY CONDITIONS IN A MOIST CHAMBER FOR VARYING PERIODS OF TIME

Exp. No.	Temperature Moist. Chamber*	Leaves Tested	Number of Hours in Moist Chamber						
			6	12–15	21–25	31–39	47–48	72–75	96
1	15°–25° C.	Number exposed to infection			234		374	304	236
		Percentage infected			36.3		23.3	8.6	10.6
2	15°–25° C.	Number exposed to infection	390	347	384	468	322		
		Percentage infected	0.0	0.9	3.1	6.8	32.3		
3	15°–25° C.	Number exposed to infection		310	185	327	424		
		Percentage infected		3.2	18.9	14.4	29.2		
4	20°–30° C.	Number exposed to infection			500		660	600	500
		Percentage infected			11.0		14.5	11.7	12.2
5	15° C.	Number exposed to infection			314		358	622	
		Percentage infected			22.9		23.7	62.7	
	25° C.	Number exposed to infection			200		532	561	
		Percentage infected			20.0		43.2	51.7	

* Plants in Experiments 1 to 4 placed in moist chamber in greenhouse (15°–30° C.). Plants in Experiment 5 placed in moist chamber in 15° C. and 25° C. incubators.

TABLE 7

RELATION OF AGE OF LEAF, AFTER EMERGENCE FROM BUD, TO INFECTION BY BASIDIOSPORES

Date (1941)	Leaves Tested	Age of Leaf in Days When Exposed to Infection														
		1	2	5	7	8	10	11	12	14	15	16	17	18	19	20–30
Jan. 12	No. exposed to infection										24	10	14	14	24	76
	Percentage infected										4.2	10.0	14.3	0.0	0.0	0.0
Mar. 20	No. exposed to infection	41		49		38		86		30			110			76
	Percentage infected	46.3		79.6		89.5		72.1		43.3			17.3			0.0
June 6	No. exposed to infection	19	2	10	2		76		16		12		16			198
	Percentage infected	31.6	50.0	60.0	50.0		7.9		0.0		0.0		0.0			0.0

leaves. The diseased leaves were counted 22 to 26 days after beginning the experiment. None of the 28 check leaves became diseased.

Penetration took place through both surfaces in all three experiments but occurred more frequently through the lower than the upper surface. The percentage of leaves becoming infected through the upper surface as compared to the lower surface in the three experiments are as follows: 13.5 vs. 31.2, 18.1 vs. 27.1, and 32.0 vs. 49.0. It is probable that the thicker cuticle on the upper surface and possibly a thick upper epidermal wall played an important part in decreasing infection.

The data indicate that the fungus may penetrate its host either directly or through the stomata.

RESISTANCE AND SUSCEPTIBILITY OF HOST VARIETIES

While making field observations on the extent and location of naturally infected leaves in 1939 and 1940, a considerable variation was noticed in the number of diseased leaves on different varieties and species of Lonicera. To determine whether differences in resistance were present under similar conditions of exposure to infection, several varieties and species of Lonicera and related genera, which had been raised from cuttings in the greenhouse, were exposed to infection with basidiospores at intervals during the winter, spring, and summer months in 1940-41. All the leaves were removed from these plants about 10 days previous to such exposure to insure each leaf being of a susceptible age. Spores were sprayed on the leaves, and the plants were examined after 19 to 30 days for the number of diseased leaves. The number of plants used varied from 1 to 14 in the five tests. Data collected from these experiments are summarized in Table 8.

Species and varieties that were consistently susceptible to infection by basidiospores were *Lonicera muendeniensis, L. prolifera, L. morrowii, L. tatarica, L. bella* (hybrid between the two preceding species), and varieties of the last two species. Those exhibiting some degrees of resistance were *L. dioica, L. gracilipes,* and *L. sempervirens. L. japonica halliana* never was infected during these (Table 8) and four other trials, and no infection ever was observed on several plants growing in the open near other susceptible species. An interesting contrast to the apparent immunity of *L. japonica halliana* was the susceptibility of a member of a different genus, *Symphoricarpos albus* (Snowberry). This plant was infected in three experiments listed here and in another one not listed. The diseased leaves developed typical basidia and basidiospores on the lower surface when placed in a moist chamber. It never has been found naturally diseased, however. The closely related species *S. orbiculatus* (Coralberry) was not infected in any of the experiments.

DISCUSSION

As a result of these investigations, some rather interesting facts concerning the life cycle and pathogenicity of *Herpobasidium deformans*

TABLE 8

RELATIVE SUSCEPTIBILITY OF SPECIES AND VARIETIES OF LONICERA, CORNUS, SYMPHORICARPUS, AND VIBURNUM

Name of Plants	No. of Trials	No. Leaves Exposed to Infection	No. of Leaves Infected	Percentage Leaves Infected
Lonicera bella	3	486	253	52.0
Lonicera bella atrorosea	5	434	99	22.8
Lonicera bella candida	5	762	346	45.4
Lonicera diuica	2	230	20	8.7
Lonicera gracilipes	5	534	20	3.7
Lonicera japonica halliana (D.) Nichols	3	690	0	0.0
Lonicera maacki	1	18	2	11.1
Lonicera minutiflora	5	998	309	31.0
Lonicera morrowii	5	1,080	397	36.8
Lonicera muendeniensis	5	1,149	522	45.4
Lonicera notha carnea	4	443	116	26.2
Lonicera prolifera	3	267	106	39.7
Lonicera prostrata	2	175	60	34.3
Lonicera sempervierns	5	519	32	6.2
Lonicera tatarica	5	809	278	34.4
Lonicera tatarica augustifolia	4	448	93	20.8
Lonicera tatarica fenzlii	5	406	160	39.4
Lonicera tatarica pallens	3	603	130	21.6
Lonicera tatarica latifolia	2	100	23	23.0
Cornus stolonifera Mich.	1	30	0	0.0
Symphoricarpos albus	5	1,265	30	2.4
Symphoricarpos orbiculatus Moench	5	1,620	0	0.0
Viburnum spp.	3	89	0	0.0

have been found. This fungus is one of the few members of the Auriculariaceae known to occur as parasites on flowering plants, although the other members of the genus, *H. filicinum* and *H. struthopteridis*, attack ferns. The connection between the basidial stage and *Glomerularia lonicerae* has been postulated, although the lack of infection resulting from the conidia of the latter and the fact that basidia have not been grown in artificial culture still leave some room for doubt. The similar binucleate mycelium of both spore stages and the fact that cultures from platings of single basidiospores, masses of basidiospores, conidia, and fragments of infected leaves all produced the conidial stages indicate that they all are part of the same fungus, and the basidiospores and conidia are stages of one life cycle.

The failure of the conidia to produce infection and the differences in germinability of the outer and inner conidia in the cyme raise a problem of the function of these structures. Martin (18) described conidia in *Platygloea peniophorae* B. and G. a closely related member of the Auriculariaceae. His statement (p. 690) follows: ". . . the groups of basidia tend to occur in loose, one-sided, cymose clusters. Some of the basidia in such clusters fail to develop fully and become transformed into conidiophores which produce irregularly globose, nearly sessile conidia." His figures show a

strikingly similar structure to the conidiophore and conidia of Glomerularia. Buddin and Wakefield (4) report a parallel situation in *Helicobasidium purpureum* (Tul.) Pat. and *Rhizoctonia crocorum* (Pers.) C. which they postulate as parts of the same cycle. In their fungus the conidial strain was less virulently parasitic than the sterile strain. Possibly these structures in *Herpobasidium deformans* are also degenerate basidia, as suggested by Martin for the similar structures of Platygloea.

SUMMARY

The causal agent of honeysuckle leaf blight is described as a new species of the genus Herpobasidium, named *H. deformans*.

The disease caused by this parasite appeared in the spring on the early leaves, and secondary infections usually followed during the balance of the year. Diseased leaves were brownish-black and often rolled and twisted. The first sign of the fungus was a thin white layer of basidia and basidiospores on the lower surface, often followed by a white powdery mass of conidia.

The host range was extended to include 33 species and varieties of Lonicera and a member of a related genus, *Symphoricarpos albus*.

The disease occurred in the northeastern and north central parts of the United States and adjacent areas of Canada and Newfoundland.

The pathogen was cultured on artificial media by platings from basidiospores, conidia, or infected leaf fragments. The mycelium, white to a very pale tan in mass, was hyaline, septate, occasionally branched without clamp connections and ranged in diameter from 1.4–3.7 μ with an average of 2.2μ. Basidia were never found on mycelia in artificial media but conidia developed from all types of isolates, including single basidiospores. The viability of mycelium in culture decreased rapidly upon drying or exposure to warm temperatures, such as 33°C.

Mycelium was at first intratracheid, later becoming both inter- and intracellular within all parts of the leaf. Some hyphae gave rise to peculiar hyphal masses within epidermal cells. The hyphae which protruded above the leaf surface from such masses never were observed bearing attached spores.

Basidia, emerging through stomata, were cylindrical and erect at first, later becoming recurved and transversely septate into four cells. They measured 2.3–5.8 μ x 14.0–35.1 μ. The basidia bore four sterigmata measuring 1.9–4.7 μ x 7.0–16.4 μ and gave rise to spores measuring 5.2–7.5 x 8.9–12.9 μ.

Basidiospores germinated by germ tubes or sporidia. Secondary spores similar in shape but smaller than basidiospores often developed on sterigma-like outgrowths from the basidiospores and germinated by tubes or sporidia.

Conditions favoring basidiospore germination were relative humidities of 99–100 per cent and temperatures of 18 to 26°C. with a minimum and maximum of about 1 and 33°C., respectively.

Conidia often formed in or adjacent to areas of basidial formation on similar mycelia. They likewise emerged through stomata and were borne on branched conidiophores which ranged in size from 2.7–5.1 μ in width at the top, 5.8–8.5 μ in width at the bottom, and 30.6–54.4 μ in length. The spores, formed in clusters of six in three pairs, were all hyaline, verrucose and binucleate. Two pairs were spherical and borne on stalks, whereas the other two spores were elongated, sessile, and produced at the junction of the two stalks. The outer pair measured 10.2–17.0 μ, the inner 8.5–13.6 μ and the elongated 5.1–13.6 μ x 11.9–22.1 μ.

The outer pairs were almost invariably the only spores that germinated, this by the formation of binucleate germ tubes. Minimum, optimum, and maximum temperatures for conidial germination were about 2°, 22–25°, and 33–40°C., respectively. Exposure to light decreased germination.

Infection of host plants followed exposure to basidiospores but never to conidia. Conditions favoring infection were temperatures of 15 to 18°C., relative humidity near or at 100 per cent, sustained periods of high humidity for 2 or more days, and the use of young leaves and of lower leaf surfaces. Minimum and maximum temperatures for infection were approximately 1°C. and 24–28°C., respectively. There was a considerable variation in susceptibility of different varieties. The variety *Lonicera japonica halliana* appeared to be immune.

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A FACTORIAL EXPERIMENT TO LEARN THE EFFECTS OF FOUR ANDROGENS INJECTED INTO MALE CHICKS[1]

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The chick comb test for androgen assay recently has excited critical investigation. It seems fair to say that with improved techniques this method is reasonably successful. But little is known about the manner in which androgenic substances interact in vivo. The thyroid gland must play an important role in controlling utilization, and perhaps intermediate compounds are formed in the body when male hormones are metabolized. We know very little concerning the action of male hormones on the thyroid gland, but we do know that the thyroid secretion modifies the reaction of the comb to male hormones. Comb growth in the chick offers some decided advantages for an analysis of such interactions, because of its responsiveness to small amounts of hormones and the accuracy with which determinations of growth can be made.

The injection of certain androgens in the male chick at relatively low dosage levels produced a decrease in testes weights without apparently decreasing the amount of endogenous male hormones (2). It was noted, however, that similar dosages of dehydroandrosterone and androstenedione and higher dosages of testosterone-propionate apparently had greater inhibitory effect on gonad weights accompanied by a reduction in the amount of androgen secreted by the testes. These substances, along with testosterone, seemed to be suitable for an investigation of possible interactions in their effects on the comb and testes of the chick.

Testosterone (*a*) is apparently the substance normally formed by the testes. Testosterone-propionate (*b*) is an ester formed from it which has proved especially efficacious in increasing androgenic action. This substance seems to be absorbed more slowly than testosterone, hence a given injection acts over a longer period of time. In addition there is probably less of this hormone excreted by the kidneys because its concentration in the blood may never become as high as that of testosterone. Dehydroandrosterone (*c*) is an androgen naturally occurring in the urine, variously estimated to have from 1/10 to 1/20 of the androgenic activity of testosterone; it also has some folliculoid activity (5). Androstenedione (*d*) a synthetic androgen of intermediate position in the chemical series,

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is especially interesting because it has about twice the male hormone activity of dehydroandrosterone and in addition has folliculoid, gonad-stimulating, and progestational action. Because of these various actions, it was hoped that a study of interactions might lead to some hypotheses about the manner in which the hormones function in the body of the male chick.

Since the factorial experiment (8) is designed to evaluate the effects of several factors, such as hormones, not only severally but in all combinations, it seemed the best adapted design. It enables one to compare the joint effect of two hormones with the effects of the two acting separately. Statistically, this is referred to as interaction.

A four-factor experiment was set up, using the hormones described above in the characteristic 16-treatment combinations, including a lot injected with only sesame oil in which the hormones were dissolved. White Leghorn male chicks were injected daily. Both alone and in combination, each factor was administered at the rate of 50 gamma per diem.

In the conduct of the experiment, each of the 16 treatment combinations was applied to from 10 to 13 chicks. The treatment of each chick was indicated by a head stain, all the birds being housed together in two cages. This prevented the confounding of treatment and environmental effects, such as is always possible when the treatment groups are kept in separate compartments. Although there were two cages, there is no evidence of cage differences.

The occurrence of unequal numbers of chicks in the groups complicates the estimates of treatment effects, as well as the tests of significance. Yates (6) has pointed out that, with disproportionate subclass numbers, the 2^n factorial experiment can be completely analyzed, using the unweighted means of the groups if interaction is present or fitting constants for the main effects if interaction is assumed negligible. Since fitting constants involves extra labor, we examined the efficiency of using the simpler method of unweighted means, even in the absence of interaction. In this particular experiment the small disproportion in subclass numbers caused no appreciable decrease in efficiency when evidence of interaction was lacking. We therefore used the unweighted group means throughout the factorial analysis of the experimental results.

It seems reasonable to present changes in hormone expression as percentages, especially along the steeper portions of the effect curves; hence the logarithms of the comb and testes weights were used as variates. This was convenient statistically for the following reason: If measured in grams, both variances and regressions on body weight differed significantly among the treatment groups, whereas the variances of the logarithms were homogeneous, as were the regressions on body weight in the case of the gonad data. In the present experiment, the transformation to logarithms affected conclusions but little.

The treatment means of body, comb, and testes weights are contained in Table 1, together with analyses of their variances.

TABLE 1

TREATMENT MEANS OF BODY WEIGHTS (GRAMS) AND OF COMB AND GONAD WIEGHTS (MILLIGRAMS)

Treatment	Symbol	No. Chicks	Means			Adjusted Means of Logarithms	
			Body	Comb	Gonad	Comb	Gonad
None	(1)	13	81	54	18.1	1.541	1.167
Testosterone	*a*	11	72	97	10.0	1.977	0.980
Testosterone-propionate	*b*	10	65	228	7.7	2.405	0.950
Dehydroandrosterone	*c*	11	67	56	9.2	1.727	0.974
Androstenedione	*d*	11	67	56	10.0	1.665	1.012
	ab	12	74	348	6.5	2.495	0.796
	ac	11	77	157	11.5	2.085	0.968
	ad	11	70	143	9.2	2.160	0.972
	bc	12	74	134	8.2	2.070	0.889
	bd	12	77	325	11.8	2.456	1.002
	cd	12	74	174	10.5	2.205	0.993
	abc	12	81	316	11.1	2.446	0.950
	abd	12	73	334	12.4	2.497	1.077
	acd	10	72	309	7.8	2.490	0.897
	bcd	12	77	329	9.8	2.489	0.928
	abcd	11	73	364	9.9	2.545	0.973

Analyses of variances of body weights and of logarithms of comb and gonad weights

Source of Variation	Degrees of Freedom	Mean Squares		
		Body	Log. Comb	Log. Gonad
Treatment means	15	246.5	1.150	0.1150
Chicks	167	123.1	0.0353	0.0247
$F(F_{.05} = 1.72, F_{.01} = 2.15)$		2.00*	32.61**	4.66**

* Significant at 5% level.
** Significant at 1% level.

The body weight differences are puzzling. The probability of greater divergence under the null hypothesis being tested is only 0.02, yet examination of the treatment means and of the treatment effects in Table 2 yields no clue to the causes.

The largest mean body weights occurred respectively in the control group and in the three-factor group, *abc*. The smallest body weights were

TABLE 2

TREATMENT EFFECTS ON BODY WIEGHT (GRAMS)

A....1.14	*AB*....1.19	*BD*....2.49	*ACD*..−2.71
B....1.86	*AC*....1.51	*CD*....0.31	*BCD*..−4.09*
C....1.89	*AD*..−3.24	*ABC*..−1.88	*ABCD*....3.34*
D....−0.86	*BC*....1.94	*ABD*..−3.04	

* Significant at 5% level.

in the single-factor treatments, while most of the two- and three-factor treatments resulted in intermediate weights. The treatment effects are equally inexplicable, only two high order interactions being significant. The natural conclusion seems to be one of the following: (1) The initial lot mean weights may have been different; (2) the causes of the differences among mean body weights lie outside the treatments administered; or (3) this is an example of the extra random fluctuation that is to be expected in about 2 samples per 100. In any case, we conclude that the treatments under investigation do not affect body weight to an extent that could be evaluated in this experiment. It should be remarked, however, that there is evidence in some other work that such treatments do affect body weight (3), so that these experimental results cannot be regarded as conclusive.

Among the chicks within the lots, there is some correlation between body weight and both comb and testes weights. Consequently, the precision of this experiment is evaluated by eliminating from error the portion of variance attributable to regression. The increase in precision due to this adjustment is 23.4 per cent in the comb series and 35.9 per cent in the gonad. Furthermore, in the gonad series there is a positive correlation, significant at the 3 per cent level, between the lot means of body and testes weight. The body weight relations are exhibited in the diagrams of Figure 1. The treatment means used in the further discussion of this experiment are adjusted by the respective regressions, comb weight, and gonad weight on body weight within the lots. The tests of significance of the differences among the two sets of adjusted means are shown in Table 3. In making the test on adjusted comb weights, the average regression within lots was

TABLE 3

TESTS OF SIGNIFICANCE OF DIFFERENCES AMONG ADJUSTED LOGARITHMIC MEANS OF COMB AND TESTES WEIGHTS

Source of Variation	Degrees of Freedom	Mean Square	
		Comb	Testes
Adjusted means	15	1.0989	.08026
Error	166	.02701	.01582
$F(F_{.01} = 2.15)$		40.69**	5.07**

** Significant at 1% level.

used, despite the fact that the lot regressions differ significantly. This point will be discussed later.

Some essential features of the experimental results are displayed in Figure 2. The weight of the testes was depressed by all treatments, while comb weight was augmented. With one exception, treatments including *b* produced about the same comb weight, the average comb weight in these eight lots being more than seven times as great as that of the check lot.

Seemingly, the 50-gamma dose of testosterone-propionate was, under

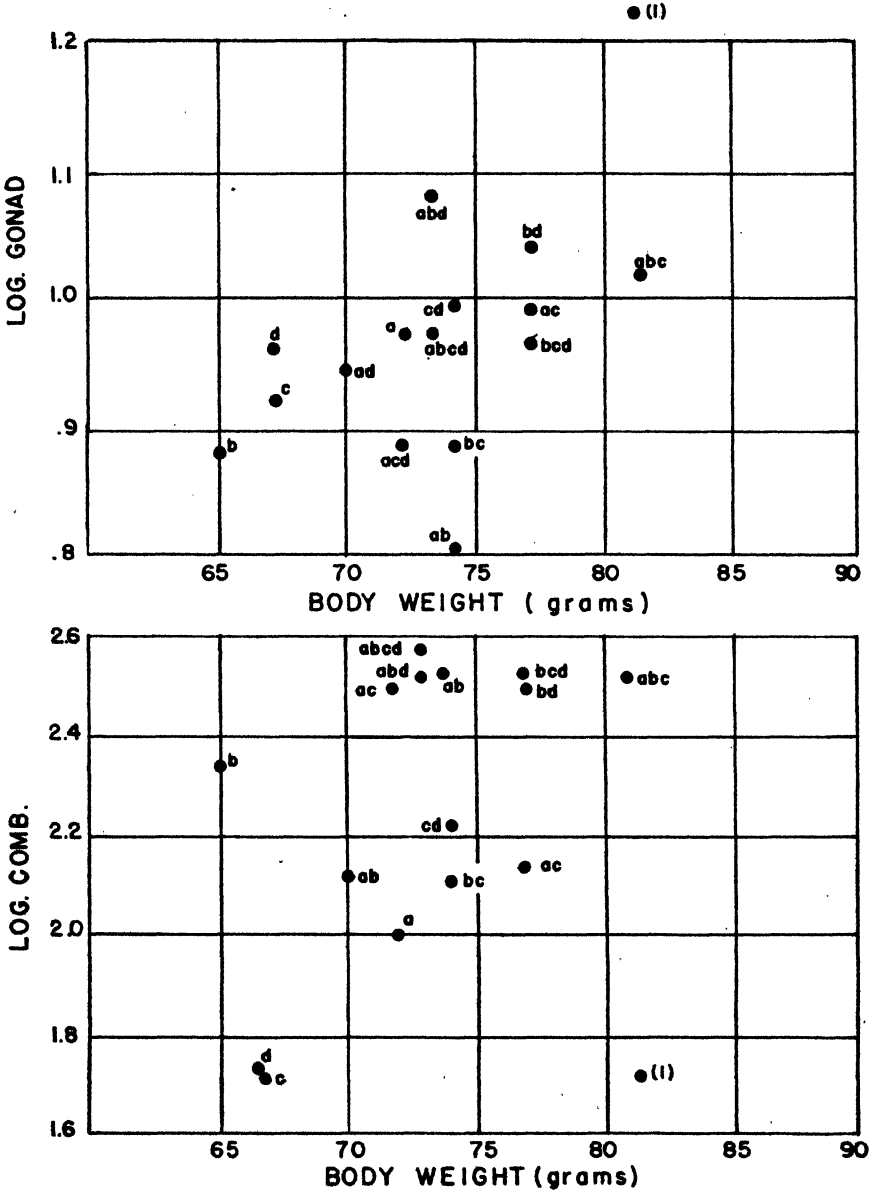


Fig. 1. Regressions of log. gonad and log. comb on body weight.

the conditions of this experiment, near the optimum for comb response, allowing little opportunity for assessing additional effects of combinations with the other hormones. If this experiment were repeated, it might be wise to reduce the dosage of *b*, seeking a level somewhat nearer its threshold value.

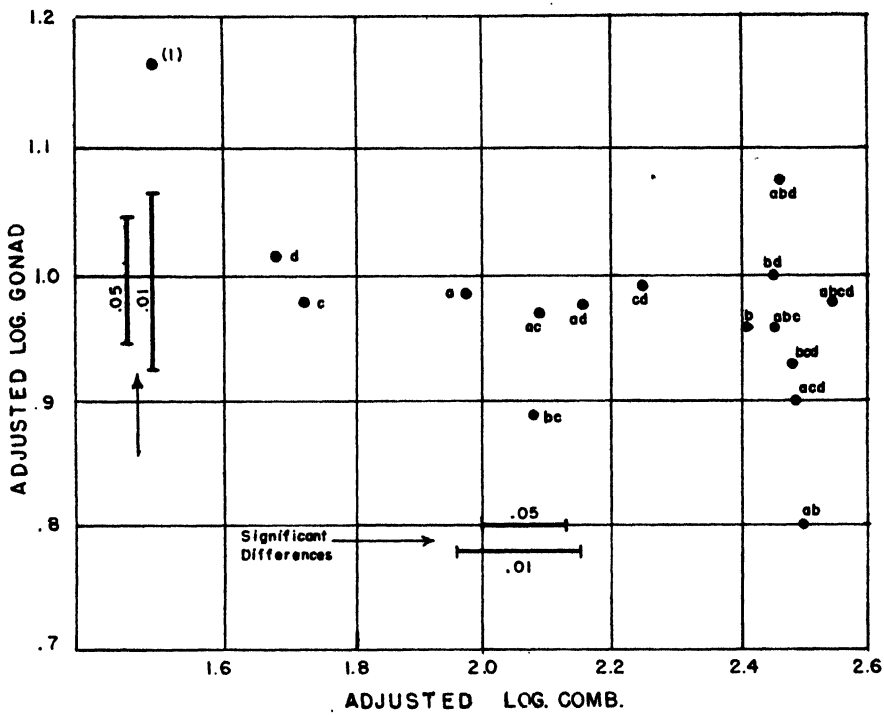


Fig. 2. Adjusted treatment means of log. comb and log. gonad.

As for *c* and *d*, their dosages were nearer threshold values for comb stimulation but like the other two hormones were more effective than desirable in decreasing gonad weights 36 per cent. The corresponding effects of androstenedione were 33 per cent and 30 per cent.

The effect of testosterone (*a*) alone was to augment comb weight by 173 per cent and depress the weight of the testes 35 per cent. That is, *a* was much more effective than either *c* or *d* in stimulating comb growth but produced about the same amount of depression of gonad weight as the other three androgens.

The appropriate combinations of dosage levels in this type of experiment would seem to require further investigation.

The *a*, *c*, and *d* treatment combinations form an interesting series in comb weights but are only slightly differentiated in their effects on the testes. This is unfortunate, because it is in possible relationships between comb and testes effects that we hoped to find clues to physiological processes. The reversed situation exists among the *b*-treatments, since in them it is the comb weights that are undifferentiated (*bc* constitutes an exception). Thus, the experiment has proved disappointing in the amount of physiological information contained.

The *bc*-series [(1), *b*, *c*, *bc*] illustrates the kind of information we were hoping for. Since *b* and *c* reduced the weights of the testes about

equally, the comb effects, $c - (1) = 1.727 - 1.541 = 0.186^{**}$ and $b - (1) = 2.405 - 1.541 = 0.864^{**}$, measure the abilities of these androgens to stimulate comb growth in spite of a perhaps slight decrease in the normal secretion of the testes. The combined treatment, *bc*, further reduces the gonad weight with a presumable further decrease in its hormonal secretion, and this may account for the resulting intermediate comb weight, despite the potential combination effect of the two injected hormones.

TABLE 4

EFFECTS OF TREATMENTS IN THE *bc*-SERIES

B-Effects			*C*-Effects		
	Comb	Gonad		Comb	Gonad
B alone, $b-(1)$,....	0.864^{**}	-0.217^{**}	*C* alone, $c-(1)$,....	0.186^{**}	-0.193^{**}
B with *c*, $bc-c$,....	0.343^{**}	-0.085	*C* with *b*, $bc-b$,....	-0.335^{**}	-0.061
BC (difference/2)...	-0.260^{**}	-0.066	*BC* (difference/2)..	-0.260^{**}	-0.066

** Significant at 1% level.

The arithmetic is set up more formally in Table 4. One-half the difference between the *B*-effects with and without *c* is the *BC* interaction. It may equally be calculated as one-half the difference between the *C*-effects with and without *b*. An interaction is negative if the effect of the combination, $bc - (1)$, is less than the sum of the effects of *b* and *c* alone. It is the negative comb interaction, *BC*, that may be attributable to failure of the combined stimuli of *b* and *c* on the comb to compensate for the decreased supply of the naturally supplied hormone. We hesitate to offer this as evidence, because it is perhaps an exceptional fraction of our data. It does, however, illustrate the possibilities of the factorial experiment.

The *acd*-series (Table 5) affords some striking interactions in the comb data, but there are no corresponding gonad effects on which to base

TABLE 5

C-EFFECTS AND INTERACTIONS IN THE *acd*-SERIES

	Comb			Gonad		
	Treatment Means	*C*	*CD*	Treatment Means	*C*	*CD*
(1)....	1.541	0.186^{**}	0.177^{**}	1.167	-0.193^{**}	0.087^{*}
c....	1.727			0.974		
d....	1.665	0.540^{**}		1.012	-0.019	
cd....	2.205			0.993		
a....	1.977	0.108	0.111^{**}	0.980	-0.012	-0.032
ac....	2.085			0.968		
ad....	2.161	0.330^{**}		0.972	-0.075	
acd....	2.490			0.897		
Average effects.....		0.291^{**}	0.144^{**}		-0.075	0.022

* Significant at 5% level.
** Significant at 1% level.

interpretations. One observes that the *C*-effects with *d* are much greater than those without, and that this phenomenon is little affected by the presence of *a* despite the fact that the *a*-treatments have uniformly higher comb weights. That is, the *CD* interactions, both alone and with *a*, are positive. One speculates about the nature of the effect of *c* and *d* on the comb when injected together, with no corresponding effects on the gonad weights. A possible clue will be discussed later.

At the moment, let us express the *C* and *D* comb effects of Table 5 in terms of percentages. The logarithm 0.186 indicates that the *c*-treatment mean is 154 per cent of check, while 0.540 shows that the mean for *cd* is 347 per cent of the *d*-mean. Now 0.177 is the logarithm of the square root of the ratio of these percentages; that is, the logarithm of $\sqrt{347/154} = 150$ per cent. Thus the effect of the combination, *cd*, expressed in milligrams, is 50 per cent greater than the combined effects of *c* and *d* administered to separate lots.

It was noted earlier that androstenedione (*d*) is reported to be gonad-stimulating. The *D*-effects in our experiment are shown in Table 6. As in the cases of the other androgens, the effect of *d* alone is to depress the weight of the testes but in a lesser degree. Two other of the *D*-effects

TABLE 6

D-EFFECTS AMONG ADJUSTED MEANS OF LOGARITHMS OF GONAD WEIGHT

	Treatment Means	*D*	*AD*	*ACD*	*ABCD*
(1)	1.167	−0.155**	0.074*	−0.060*	−0.001
d	1.012				
a	.980	−0.008			
ad	.972				
c	.974	0.019	−0.045		
cd	.993				
ac	.968	−0.071			
acd	.897				
b	.950	0.052	0.114	−0.061*	
bd	1.002				
ab	.796	0.281**			
abd	1.077				
bc	.889	0.039	−0.008		
bcd	.928				
abc	.950	0.023			
abcd	.973				
		0.022	0.034*	−0.061**	−0.001

* Significant at 5% level.
** Significant at 1% level.

are negative, but five are positive, indicating a slight tendency of *d* to increase weight. But if Table 5 is re-examined in the light of this hypothesis, and if stimulation of secretion of the male hormone by the testes is thought of instead of weight, then the notable comb effects of c in the presence of *d* could be attributed to increased production of hormone by

the testes. Why this should occur in the presence of *c* but not in its absence is still a question.

Turning now from the biological to some of the more formal statistical features of the experiment, it may be well to note in review the following points: (1) The logarithm transformation was used in the comb and gonad weights partly because it seems reasonable to express in percentages the changes occurring in some regions of the effect curve. The transformation was advantageous statistically, because the lot variances were correlated with their means and because the lot regression of comb and gonad on body weights became erratic with higher dosages. The logarithms had homogeneous variances and regressions in the gonad series, but were still somewhat heterogeneous in the comb data. (2) After computation of the deviations from error regression and of the variance of lot means, the disproportion among the subclass numbers of chicks was ignored, the unweighted lot means being used. It was found in this experiment that no appreciable loss of efficiency was incurred. (3) While there were significant differences among the means of the body weights, they seemed to be unrelated to the treatments. Hence all means of logarithms of comb and gonad weights were adjusted to the average body weight, and these adjusted means were used in the factorial analysis. (4) The object of the experiment was to relate the treatment effects on comb and gonad weights and to make inferences about the actions of the experimental hormones within the body of the chick. The separate comb and gonad series, therefore, were of only secondary interest.

The statisticians will have observed our failure to use any of the elegant methods available for computing the average effects in factorial experiments. Examination of our data showed us promptly that the averages of the effects tended to conceal the information we sought. With the exception of the body weight average effects in Table 2, we found not a single one that was helpful. Table 6 illustrates the situation that confronted us, whereas the partial set of comb effects in Table 5 seems to represent the kind of regular behavior that makes average effects useful. Our data demanded the kind of analysis explained by Yates on pages 9 to 13 of his "The Design and Analysis of Factorial Experiments."

Two remarks remain to be made about tests of significance. Since testing has played no important part in our investigation, we have been content to ignore some biases. Those in the gonad series could be handled rather easily by the methods of Yates (7) or Cochran (see Parker's (4) citation of Cochran). We calculated the correction for the treatment mean square in a few instances where the level of significance was near 5 per cent but found it beyond the significant digits being carried in the computations.

In the covariance analysis of the comb weight logarithms, pronounced differences were found among the treatment regressions, and consequently, adjustments to mean body weight were made by use of individual regressions. The variance of treatment mean differences is, therefore, the sum of the variances of both the means and the regression coeffi-

cients involved. For all the average effects of any one set of treatments, this common variance is easily calculated. In the presence of 16-treatment experiment, it is an average of 32 variances, all readily available from previous computations. In the error used for the main effects, we found the bias 1.6 per cent; but since we found none of our conclusions were affected by the more accurate calculation of the variance, we did not compute it for the various partial effects.

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EFFECTS OF DIFFERENT FOOD PLANTS ON EGG PRODUCTION AND ADULT SURVIVAL OF THE GRASSHOPPER, *MELANOPLUS BIVITTATUS* (SAY)[1]

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Grasshoppers are usually rather lively insects, but it is possible that, in large acreages of corn, alfalfa, clover, and other cultivated or wild plants, certain 'hoppers might remain within one food locale for nearly all or a greater part of their lifetimes. Should such a possibility occur, what effects might an unvaried diet have on the grasshoppers' biological responses and potentials? Such speculation becomes more interesting since man-made environments of large fields of succulent food, especially suitable to grasshoppers, may set up excellent ecological conditions for building larger populations than might have resulted from the same number of grasshoppers on their natural, wild food plants.

Uvarov (20), in his handbook on locusts and grasshoppers, has pointed out that almost every injurious species has a long list of host-plants. Nevertheless, the preferred natural food "is Graminaceous plants." Both in species and individuals, he notes that grasshopper associations are most numerous in habitats with a predominance of grasses. Parker (10) writes, "Previous to the growing of crops, native grasses were the main source of food for grasshoppers." Recently, however, Isely (7) published his conviction that the idea of grasshoppers eating mainly grasses "is no longer tenable." Vestal (21) surveyed insects in several forest habitats and found only one grasshopper, *Melanoplus islandicus,* restricted to the tree itself; some other species were found in transitional forest margins. In Uvarov (20), Parker (11), and Isely (7) are many other references to the eating habits of grasshoppers.

Man's agricultural practices have established microclimates—some unfavorable to grasshoppers and locusts, others especially suited to and stimulatory to their multiplication up to injurious population levels. Uvarov (20) mentions the apparent connections between alfalfa culture in both North America and Russian Turkestan and the regular increase in the numbers of certain grasshoppers. Abundance of good food offered by alfalfa and the practice of leaving the soil undisturbed for at least 2 years give exceptionally suitable environmental surroundings for some 'hopper species. Unplowed fields offer excellent beds for egg-laying. In addition,

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the absence of cultivation eliminates the usual injury inflicted on the eggs by plowing, discing, harrowing, and other soil preparation practices.

Several more recent references have similar notations regarding relationships between farm practices and grasshopper abundance. Sanderson (15) writes that when Arkansas was predominantly a cotton-producing area, prior to 1932, grasshoppers were not a major problem. After 1933 there was a marked reduction in the cotton acreage, with five-eighths of the old cotton land planted in soybeans and most of the remainder in corn, legumes, or pasture land. Also, by 1936, widespread increase in grasshoppers necessitated systematic control procedures. It should be recalled, however, that these changes in crops came during that same climatological period associated with great upswings in numbers of grasshoppers. Coactions with climate and crops, as well as other factors, probably contributed to the situation.

Again referring to Arkansas, Isely (6), in 1942, points to the increase of *M. differentialis* and remarks that outbreaks first occurred where alfalfa production had been established. Previously, this species "was at worst only a local pest." He is also of the opinion that introduction of soybeans was "undoubtedly . . . responsible for the change in status of this grasshopper."

Shotwell (16, 17), too, discusses the changes brought about by agriculture in areas of natural vegetation in relation to grasshopper populations. Annand (1) offers a more general article on the difficulties involved with grasshoppers and other insects, as agricultural methods have changed in attempts to resist the effects of drouth.

In view of the implications in the above observations and discussions, it was decided to compare, under controlled laboratory conditions, a typical adult grasshopper's ability to live and reproduce while its diet was restricted to a single food plant, with similar abilities exhibited in parallel experiments with a mixed diet.

MATERIALS AND METHODS

Specimens of *M. bivittatus* (Say) were selected as the experimental insects because they could be obtained in large enough numbers; because they are of economic importance in Iowa; and because little precise information was available on the interaction of food and egg-formation in this or any other species of grasshopper.

Initiation of experiments, selection of grasshoppers, and the manner of cage operations were as described elsewhere by Drake, Decker, and Tauber (3), except that only the smaller cages, with five pairs of grasshoppers in each, were used; and only one variety of food was available in each cage throughout the experiment. All of these cages were under roof in the screened insectary at Ames.[3] All manipulations, observations,

[3] This screen-house is a structure whose sides consist only of screen-wire, except for uprights supporting the roof and the frames of the doors. The peaked roof is covered with cedar shingles; eaves are narrow to allow entry of as much light as possible. Inside the building are greenhouse benches on which the insect cages were placed. The entire arrangement provides a light, airy surrounding in which observations and manipulations may be made without interference by weather conditions.

and determinations of data were performed by the senior author in order to eliminate any variations in technique. Of the results, included in another publication, on oviposition and longevity of *M. bivittatus* on a mixed diet (3), only data from the smaller cages inside the screen-house are used in this paper for comparison as "controls."

Field-collected specimens were used rather than 'hoppers reared from eggs under artificial conditions in constant temperature, since Parker (9) found the latter individuals were not so vigorous nor productive in his experiments with *M. mexicanus*.

Experiments extended through the summers of 1938, 1939, and 1940. Ten cages (50 pairs) of controls on a mixed diet were maintained in 1938; 20 cages (100 pairs) in 1939 and again in 1940.

Wild lettuce, wild mustard, great ragweed, and hemp were gathered fresh each day along roadsides near Ames, plunged in water containers, and transported to the insectary. The cottonwood, willow, and mulberry leaves were on young tender shoots from trees near the screen-house. Onion plants, oat and wheat sprouts, castor-oil plants, and small pumpkin and watermelon vines were supplied in pots and replaced as needed. All other plant materials were available in the insectary gardens and grounds. A complete list of tested food plants, with scientific names, will be found in Table 1. A few additional cages were run as a preliminary exploration in the field of water needs by grasshoppers. Cages, each with five pairs, were set up with a large amount of fresh-cut alfalfa, corn, or timothy which was not placed in water, but was left to wilt and become drier and drier as the summer progressed. In these cages the plant material was never replaced, and was the sole source of solid food as well as moisture. Other cages were begun with alfalfa already dried to brittleness but with shallow dishes of fresh water always available.

RESULTS AND DISCUSSION

Thirty different fresh plant materials were tested during the three summers. The number of pairs of *M. bivittatus* used with each plant varied considerably, and depended largely on the availability of the specific food item—especially if it were a wild plant which had to be collected daily along roads or in fields within a reasonable collecting distance around Ames. The maximum number of pairs was 100 on young corn plants; the minimum, 20 pairs on ripe tomato. Seventeen of the 30 foods were tested on 50 or more pairs; 12 plants on 25 to 50 pairs; and only 1 (tomato) on as few as 20 pairs.

When data for the 3 years are summarized, and the tested plants arranged according to average number of eggs produced per female, the order is as shown in Table 1, with highest egg production at the top of the column.

Data in Table 1 indicate that wild lettuce and alfalfa possess almost identical properties in being suitable foods for ovipositing females of *M. bivittatus*. Although those females on alfalfa tended to live a little longer (3.1 more days), they averaged one less egg per female (140) than those on wild lettuce (141). Third and fourth are red clover and garden

TABLE 1

SUMMARY OF THREE YEARS' DATA ON EGG PRODUCTION AND SURVIVAL OF *M. bivittatus* (Say) FEMALES ON DIFFERENT FOODS

Food Plant*	No. Pairs Tested	Total No. Eggs Produced	Total Egg Pods Deposited	Ave. No. Eggs Per Pod	Ave. No. Pods Per Female	Ave. No. Eggs Per Female	Ave. No. Days Survival Per Female After Onset of Oviposition	Ave. No. Eggs Per "Female-Day"
Wild lettuce plant (*Lactuca* sp.)	65	9,180	124	74	1.90	141	20.5	6.9
Alfalfa plant (*Medicago sativa*)	75	10,505	138	76	1.84	140	23.4	6.0
Red clover plant (*Trifolium pratense*)	85	9,701	127	76	1.49	114	23.5	4.9
Garden leaf lettuce (*Lactuca* sp.)	80	8,971	106	85	1.32	112	23.5	4.8
Garden onion leaves (*Allium* sp.)	65	6,199	78	78	1.20	95	25.4	3.7
Soybean plant (*Glycine* sp.)	55	4,862	65	75	1.18	88	19.8	4.4
Sweet clover plant (*Melilotus* sp.)	70	5,264	77	68	1.10	75	19.0	3.9
Sudan grass leaves (*Sorghum vulgare* var. *sudanense*)	35	2,552	35	73	1.00	73	22.9	3.2
Corn plant (*Zea mays*)	100	6,644	95	70	0.95	66	20.4	3.2
Cabbage leaves (*Brassica oleracea*)	70	4,222	65	65	0.92	60	17.5	3.4
Amber cane plant [*Sorghum vulgare* (*Andropogon sorghum*)]	50	2,893	39	74	0.78	58	22.0	2.6
Timothy plant (*Phleum pratense*)	35	1,886	29	65	0.82	54	22.1	2.4

(*Table 1—continued*)

Wild mustard plant (*Brassica* sp.)	30	1,602	26	62	0.86	53	15.2	3.5
Oat sprout (*Avena sativa*)	75	3,882	60	65	0.80	52	19.3	2.7
Great ragweed plant (*Ambrosia trifida*)	50	2,428	38	64	0.76	49	16.2	3.0
Corn silk (*Zea mays*)	35	1,671	34	49	0.97	48	27.1	1.8
Quackgrass plant (*Agropyron repens*)	50	2,285	34	67	0.68	46	20.2	2.3
Pumpkin plant (*Cucurbita pepo*)	50	2,258	42	54	0.84	45	17.2	2.6
Wheat sprout (*Triticum aestivum*)	45	1,954	29	67	0.64	43	18.2	2.4
Watermelon plant (*Citrullus vulgaris*)	25	1,086	17	64	0.68	43	19.4	2.2
Cottonwood shoots (*Populus deltoides*)	30	1,263	29	44	0.96	42	27.1	1.5
Hemp (*Cannabis sativa*)	50	2,039	36	57	0.72	41	12.3	3.3
Willow shoots (*Salix* sp.)	60	2,392	46	52	0.76	40	17.4	2.3
Crabgrass plant (*Digitaria sanguinalis*)	35	1,230	23	53	0.66	35	20.5	1.7
Mulberry shoots (*Morus rubra*)	35	1,161	21	55	0.60	33	9.6	3.4
Corn tassel (*Zea mays*)	35	1,151	29	40	0.82	33	22.9	1.4
Meadow foxtail grass (*Alopecurus pratensis*)	75	1,698	34	50	0.45	23	12.8	1.8
Sudan grass seedhead (*Sorghum vulgare* var. *sudanense*)	30	696	16	44	0.53	23	17.5	1.3
Ripe tomato (*Lycopersicon esculentum*)	20	387	9	43	0.45	19	18.3	1.0
Castor-oil plant (*Ricinus communis*)	40	701	15	47	0.37	18	12.2	1.5

* Scientific names from Gray (4) and Hitchock (5).

leaf lettuce with average eggs per female of 114 and 112, respectively. Fifth place was taken by females feeding on onion plants, with an average of 95 eggs per female. Soybean was sixth with 88 eggs per female; sweet clover, seventh, with 75 per female. From this position there was a more gradual and almost regular reduction in egg production through the remaining 23 food plants. Thirtieth, and last, in rank was the castor-oil plant with an average of 18 eggs per female.

Isely (7) classifies *M. bivittatus* among the "mixed feeders" which naturally feed on forbs, but will eat grasses and other wild and cultivated plants when coarser weeds are not available. Wild lettuce (*Lactuca* sp.) is included among the forbs, and its position in Table 1 points up its importance in the eating habits of the two-striped grasshopper. The writers have not had access to the "progress reports" of Pfadt, referred to by Isely (7). Pfadt (12, 13) is reported as finding alfalfa "one of the first choice foods" for eight species of Wyoming grasshoppers, all Cyrtacanthacrinae. This finding is of interest in view of alfalfa's effect on the fecundity of *M. bivittatus*.

When results from the mixed-diet grasshoppers ("controls") of Table 2 are compared with those from the single food plant experiments of Table 1, it is evident that either wild lettuce, alfalfa, red clover, garden lettuce, or onion plant, as separate foods, is associated with better egg production than is exhibited by ovipositing grasshoppers on a mixed diet. Wild lettuce and alfalfa are particularly striking with records of approximately 140 eggs per female, as compared with an average of 91 eggs for females on the mixed diet. These results point to rather clear-cut influence of the better separate foods as well as the mixed diet on egg formation alone, for there seems to be no significant difference in the days of survival by the females after oviposition had begun. It should be pointed out again that these experiments were carried out on 'hoppers that had reached the adult stage before being put on these separate or mixed foods. Whether results of the same magnitude would appear with specimens of *M. bivittatus* reared on such separate foods or mixed diets throughout their whole lives is not known, although Sanderson's (15) results with *M. differentialis* indicate the differences might be even more pronounced.

The high reproductive potentials with alfalfa and red clover, especially the former, support the suggestions previously mentioned that certain crops, notably alfalfa, may be factorial in building up large grasshopper populations.

Judging from the results in Table 1, other cultivated crops often found in large acreages, such as soybeans, sweet clover, corn, timothy, and oat sprouts, as separate foods, apparently are not so likely to be correlated with a higher fecundity by *M. bivittatus*. Wheat sprouts rank nineteenth and are, in general, about half as effective as a mixed diet and about one-third as effective as alfalfa and wild lettuce. Neither mature oats nor wheat, with seed-heads, was tested; such a stage of these crops might give different results. Maltzev (8) has reported that adults of *Pachytylus migratorius* L. raised on oats do not develop genital products.

TABLE 2

SUMMARY OF DATA FROM CONTROL SPECIMENS OF *M. bivittatus* (Say) ON MIXED DIET*

Year	No. Pairs Tested	Total No. Eggs Produced	Total Egg Pods Deposited	Ave. No. Eggs Per Pod	Ave. No. Pods Per Female	Ave. No. Eggs Per Female	Ave. No. Days Survival Per Female After Onset of Oviposition	Ave. No. Eggs Per "Female-Day"
1938	50	5,061	67	75.5	1.3	101.2	25.3	4.0
1939	100	9,073	120	74.4	1.2	90.7	22.2	4.1
1940	100	8,155	120	67.9	1.2	81.5	20.7	3.9
Averages				72.6	1.2	91.1	22.7	4.0

* These data from Drake, Decker, and Tauber (3).

FOOD PREFERENCES IN RELATION TO SURVIVAL AND EGG PRODUCTION

Uvarov (20) and Shotwell (17) have noted certain food preferences of grasshoppers. The present experiments show no regular agreement between such specific preferences and egg production. In a shelterbelt in South Dakota in 1931, mulberry seemed to be the first choice as a tree-food by *Melanoplus bivittatus* (17), but mulberry leaves ranked twenty-fifth among the 30 foods tested; the average number of eggs per female was less than one-fourth the alfalfa average. Small grains are reportedly preferred to alfalfa and corn in the field (17), but in the present experiments both alfalfa and corn are better egg producers than oat and wheat sprouts. Here, again, the days of survival on these four foods do not differ significantly. Wild lettuce (*Lactuca*) is first in egg production, and was also reported (17) to be a favorite food, especially during rather dry weather. Although "a distinct preference for corn silk" has been noted (17), it is approximately only one-third as good as wild lettuce for egg production, namely, 48 to 141 eggs, respectively, per female.

Shotwell (17) also noted an aversion, on the part of *M. bivittatus*, to sorghums after the latter reach a certain stage of growth. However, when *M. bivittatus* has no other food, as was the case in one of the present trials, it will eat sorghum (amber cane) and do fairly well: eleventh in egg production and among the better examples in average days of survival. Spain (18) proved that castor-oil plant was a poor food for grasshoppers. In these experiments, two-striped grasshoppers on castor-oil plant made the poorest record (average of 18 eggs per female), and the survival time was also one of the lowest.

During the three summers of diet tests, observations on food preferences also were noted. One of the most striking selections was evidenced with blooming red clover. When this plant, with flower heads, was put in a cage, the flowers were invariably eaten first; the preference was so pronounced that a determined "belligerence" between competing feeders often was provoked. This tendency to destroy clover flower heads could be particularly detrimental if attempts were being made to produce mature clover seed as a market crop.

Blossoms on sweet clover, wild mustard, hemp, and on corn tassels were also favorite foods, but not to the degree of red clover flowers. Swain (19) has recently noted a similar damage to inflorescences by the Mormon cricket, *Anabrus simplex*. Wild lettuce, alfalfa, garden lettuce, red clover leaves, sweet clover leaves, corn plant, wild mustard, ragweed, corn silk, and hemp leaves all were eaten in large amounts as separate diet items. No particular liking for crabgrass, quackgrass, or meadow foxtail grass was observed, although "Graminaceous plants" are recorded as the preferred natural food by many grasshoppers (10, 20).

Gardeners often have come upon a grasshopper nibbling on a near-ripe tomato. This habit prompted the inclusion of ripe tomato in these tests. As an item of regular diet it was noticeably preferred, but as a stimulus to egg production tomato ranked twenty-ninth. Perhaps the 'hoppers' destructive eating of tomato may be laid to a fortuitous meeting

with an easily procured and abundant source of moisture. Perhaps, also, variations in the volume of "bleeding" from plants or differences in the water content of the tissues may explain why some plant species in a field may be eaten down into the ground, while other species are relatively untouched.

Our experience with mulberry leaves does not agree with Shotwell's (17) observation of preference as noted above in the South Dakota shelterbelt. Mulberry leaves were not readily or eagerly consumed, even on young sprouts. Mulberry furnished poor food for egg production and gave the shortest average survival time among the 30 food tests: 9.6 days, as compared with an average of 22.7 days on a mixed diet. However, after a few days with no other food than mulberry, the quantity eaten was noticeably increased, and mulberry leaves seemed to be eaten with more "enthusiasm." Mulberry leaves were consumed in perceptibly greater amounts than either willow or cottonwood leaves.

In regard to some cases of defoliation, it should be remembered that in hot, dry periods, grasshoppers "roost" in trees and shrubs to escape the heat of the baked earth. They may eat while there—a necessity of circumstance rather than a dietary choice.

In connection with preferences for certain parts of plants, it may be pointed out that whole young corn plants (8 to 18 inches high), corn silk, and corn tassels were tested separately. All three foods were eagerly eaten, but egg production was, respectively, 66, 48, and 33 eggs per female; and survival was, again respectively, 20.4, 27.1, and 22.9 days per female. Young plants of Sudan grass and Sudan grass seedheads in the green stage also were tested separately. The seedheads were not especially relished. Egg production was 73 per female for the plant as a whole and only 23 for the seedheads alone. Survival was 22.9 days per female on the entire plant, 17.5 days on its seedhead.

CONSISTENT RESULTS FROM REPEATED TRIALS

Availability of cages, collectibility of grasshoppers at the necessary stage of development, and abundance of certain foods from year to year did not make it possible to test all 30 foods during each of the three summers. However, 12 of the 30 test plants were used every summer. When these 12 are arranged for each year according to the average number of eggs produced per female, the rankings are as shown in Table 3.

Table 3 does not exhibit an absolute consistency in the ranking of the 12 foods from year to year, but the agreement in general position is remarkable, considering all the variables which could enter experiments of this type. The three seasons, naturally, were not climatologically identical; each of the foods could not be in exactly the same state of development each year. Seventeen of the remaining 18 foods were tested for 2 years; 7 of the 17 had exactly the same rank in the 2 years, or fluctuated just one position up or down in the order. Wild mustard was used only once; a nearby source was not available in the other 2 years.

The consistently poor showing of meadow foxtail grass in all 3 years

TABLE 3

DESCENDING ORDER OF FOOD PLANTS FOR THREE YEARS ACCORDING TO AVERAGE NUMBER OF EGGS PRODUCED BY FEMALES OF *M. bivittatus* (Say)

Rank	1938	1939	1940
1	Alfalfa	Wild Lettuce	Wild Lettuce
2	Wild Lettuce	Alfalfa	Alfalfa
3	Garden Lettuce	Garden Lettuce	Red Clover
4	Red Clover	Red Clover	Onion Leaves
5	Onion Leaves	Onion Leaves	Garden Lettuce
6	Corn Plant	Sweet Clover	Corn Plant
7	Cabbage Leaves	Corn Plant	Cabbage Leaves
8	Sweet Clover	Cabbage Leaves	Sweet Clover
9	Oat Sprouts	Oat Sprouts	Pumpkin Plants
10	Pumpkin Plants	Willow Shoots	Oat Sprouts
11	Willow Shoots	Pumpkin Plants	Willow Shoots
12	Foxtail Grass	Foxtail Grass	Foxtail Grass

adds another bit of support to Isely's (7) expression that certain grasses are not always the best or preferred food of all grasshoppers, contrary to some published accounts.

RELATION BETWEEN DURATION OF SURVIVAL AND FECUNDITY

Since egg production by the female of *Melanoplus bivittatus* in suitable environmental surroundings is typically not a matter of a single oviposition, the number of her life span's days available after copulation for successive egg deposits becomes an important factor in measuring her egg productiveness. To determine the exact number of days each female lived after her initial oviposition was, of course, impossible in these tests with such large numbers and with five pairs per cage. But, since all the test 'hoppers were of about the same age when collected in one small locality and had passed through the same developmental history, it is perhaps permissible to assume that the majority of the females would be ready to produce the first egg pod within a few days of each other's depositions. On this assumption, therefore, each female's potential oviposition period was measured from that day when the first egg pod was found in any of the experimental cages. The average figure for the duration of this period for females on each kind of food is recorded in Table 1. When the average number of eggs per female is divided by this average "oviposition-survival" figure, the average number of eggs per "female-day" is available. This figure also is recorded in Table 1. Is this average number

of eggs per female-day a better measure of egg productiveness than the simple average number of eggs per female? According to data accruing from these experiments, there seems to be little choice. Although there is not complete agreement between the position in rank of the two averages, one can see that, in general, higher egg production per individual usually demands a higher number of days of survival.

There are some notable exceptions to the above generalizations. For example, females feeding on corn silk alone, and cottonwood leaves alone, both had average oviposition-survival figures of 27.1 days—the highest among the 30 tests. Yet the average numbers of egg per female-day in these two cases were seventeenth and twenty-first in rank. Also, females on mulberry leaves had the shortest average oviposition-survival time (9.6 days), but their average number of eggs per female day was not the lowest.

Table 1 also presents averages for the number of eggs per pod and for the number of pods per female for each food. Here again, in both categories, there is general agreement in rank with the average number of eggs per female. In other words, females with the higher average egg records produce a larger average number of pods, and those pods tend to contain more eggs.

EFFECT OF DRY FOODS

Table 4 contains results of preliminary experiments with drying and dried foods. The samples in each case (only 10 pairs) are undoubtedly too small to warrant unqualified conclusions, but perhaps some trends may be legitimately deduced. At least it seems clear that egg production under all of these rather drastic conditions is poor, the drying timothy giving the lowest record (4 eggs per female) in all of the operations. With access to water provided in the trials with dry alfalfa, the 'hoppers apparently can do as well as on some foods which were supplied as they occur in nature (Compare Table 4 and the lower part of Table 1).

Surprisingly good records came from these dry food trials so far as average survival time was concerned. Drying corn and dry alfalfa plus water allowed the tested females to survive as long, on the average, as those females which gave the best records on succulent foods. Drying alfalfa and drying timothy, however, were not able to support life for very long; only females fed castor-oil plants exhibited a lower average survival.

It may be incidental coincidence in these preliminary trials, but egg production varied directly with each plant's resistance to drying out completely during the duration of the tests. The thick corn stalks could retain considerable moisture in the roofed screen-house, and the 'hoppers on drying corn ate large wedges into the moisture-filled stalks in preference to the thoroughly dried leaves. Similarly, the thicker stalks of alfalfa would retain more moisture than the thin timothy stems. Those females on drying alfalfa thoroughly "barked" the stems after the leaves had become dry.

TABLE 4

EGG PRODUCTION AND SURVIVAL OF *M. bivittatus* (Say) FEMALES ON FOODS DEFICIENT IN WATER

Food	No. Pairs Tested	Total No. Eggs Produced	Total Egg Pods Deposited	Ave. No. Eggs Per Pod	Ave. No. Pods Per Female	Ave. No. Eggs Per Female	Ave. No. Days Survival Per Female After Onset of Oviposition	Ave. No. Eggs Per "Female-Day"
Drying corn	10	348	6	58	0.6	35	24.4	1.4
Drying alfalfa	10	290	4	73	0.4	29	13.7	2.1
Drying timothy	10	40	1	40	0.1	4	13.7	0.3
Dry alfalfa plus water	10	382	8	48	0.8	38	23.9	1.6

NERVOUSNESS ASSOCIATED WITH WATER DEFICIENCY

Another reaction was noted in the cages with the drying foods. As the plants became more dehydrated, the 'hoppers caged thereon became increasingly restless and were much more inclined to jump and fly about forcefully and vigorously in attempts to escape from the slightest disturbance—even simply that of an observer's walking past the cages. In comparison, test 'hoppers on green food became quite "tame" and were decidedly more tolerant of the manipulations of the experiments. Could such hyper-irritability, as displayed by the thirsty 'hoppers, play a part in the migration of solitary 'hoppers by making them more receptive to some stimulus which would initiate an "explosion" of motion? Uvarov (20) is of the opinion that shortage of green food does not account for the migration of gregarious locusts which move in bands. In the case of flights by so-called solitary 'hoppers, the situation may be somewhat different. Rockwood has noted that such 'hopper flights seem to be connected with high temperatures and low relative humidity (14). Under drouth conditions, these factors would be paramount (9, 10). The irritability which seems to accompany a scarcity of succulent food, along with the heat which prevails in summer dry spells on the Great Plains of the United States, might conceivably establish conditions for setting such group flights into operation. The increased flying and jumping resulting from some stimulus—perhaps a sudden flutter of a branch, or the flight of a bird, or a gust of wind—might be sufficient to provoke the nervous pests into migratory flight.

Uvarov's view (20) that the impetus for swarming and migration is centered in an increased internal body temperature resulting from subjection to environmental warmth possibly could contribute also, nevertheless. A 'hopper with access only to dry food would tend to become dehydrated. Presumably less water vapor would be available for release in respiration, and a point must be reached eventually when the insect attempts to restrict this water loss. Bodine (2) has shown that *M. femur-rubrum* and *M. differentialis* may lose from 20 per cent to 25 per cent in body weight when fasting, and this loss comes mainly from a decrease in the water content of the specimens. Water has a high heat capacity and would be used as a "carrier" for the elimination of excess metabolic heat as well as excess warmth taken up from the environment. If this heat loss mechanism could not function because of a deficiency of evaporatable water, the internal temperature of the dehydrated 'hopper might rise to that level of irritability which Uvarov proposes in his explanation of swarming, or which the writers suggest as the basis for the extra receptiveness to stimuli which provokes flight. The higher internal body temperature, by itself, probably is not responsible for the migration phenomenon. Perhaps hyperirritability and higher body temperature fit into a chain of interactions which will weave themselves into the complete eventual explanation of massed 'hopper flights. This suggestion of the writers is, it is to be understood, only a matter of conjecture; no experimental evidence is now available.

CANNIBALISM

In those cages with drying food, cannibalism was very pronounced. Dead specimens were removed each morning and noon, but nevertheless, 32 of the 60 'hoppers in these cages suffered from the craving of their cagemates for moisture. The soft abdominal region was usually first opened; in some cases nearly the whole body was consumed. Inasmuch as the last 6 survivors (1 in each cage) could not consume themselves, the percentage of cannibalism would be based on 32 cases out of 54 deaths, giving a figure of 59.3 per cent. In the two cages with dried alfalfa plus water, no cannibalism occurred. Among the more than 3,000 'hoppers on green food, only a few bodies were even partially eaten.

COMPARISON WITH PREVIOUSLY PUBLISHED RECORDS

Shotwell (17) recounts certain information gathered from five pairs of *M. bivittatus* maintained throughout their entire lifetimes under laboratory conditions at room temperature. No mention is made of the type of food. These five females averaged 127 eggs, in comparison with an average of 91 eggs produced by the 250 females on a mixed diet used in the trials described by Drake, Decker, and Tauber (3). Only those females on wild lettuce and those on alfalfa exceeded the average of the five females studied by Shotwell. Although Shotwell does not record the number of days each female survived after the first oviposition, this information can be calculated from other data he furnishes and comes to an average of slightly more than 15 days. This figure is less than the average survival on the mixed diet investigations, namely 22.7 days (3), and less than the average survival for many of the females on single food plants (see Table 1).

In his experiments Shotwell (17) was able to determine the number of days between copulation and the first oviposition. This time averaged 18.4 days for his five specimens and ranged from 7 to 30 days. The duration of this postcopulation period would prove very important in experimental procedures such as the present ones when separate food items were tested. If it so happened that a certain unvaried item of diet was not favorable to sustain life for a sufficient length of time (averaging 18.4 days after copulation) to allow maturation of the ova, the female would die before ovipositing. This factor may be crucial in explaining the inability of certain plant foods to make high records in egg production. The females concerned would simply die before their genital products were ready to be released.

SUMMARY AND CONCLUSIONS

1. Field-collected specimens of *M. bivitattus* (Say) were used during 1938, 1939, and 1940 to test the maintenance value, as measured by egg production and adult longevity, of certain separate wild and cultivated food plants.

2. Five pairs of newly emerged adult 'hoppers were placed, before mating, in each cage (12x12x24 inches, with removable soil tray at bottom).

From the time of caging the experimental specimens until death, individuals in each cage received only one kind of food. Plants were either potted or cut and kept fresh in water. Food was always changed at least once daily; during very hot weather fresh food was offered twice daily.

3. Parallel set-ups of cages with a "mixed diet" were managed during the same seasons, and served as controls.

4. All control and experimental cages were kept under roof in an open screen-house, with access to free circulation of air currents.

5. Preliminary trials also were made to determine the effects of drying food, and dried food plus water, on oviposition and adult longevity.

6. Thirty different fresh plant materials, three drying foods, and one completely dry food plus water, were tested.

7. Nearly 1,600 pairs of 'hoppers were tested on the different separate items of diet; 250 pairs on mixed diet served as controls. The maximum number of pairs on any one food was 100 on young corn plants. The minimum number on fresh food was 20 pairs on ripe tomato. Only 10 pairs were tested on each of the drying and dry foods. Seventeen of the fresh foods were tested on 50 or more pairs; 12 plants on 25 to 50 pairs; only one on as few as 20 pairs.

8. Wild lettuce and alfalfa effected the highest egg production with an average of 141 and 140 eggs, respectively, per female. Other high producers were red clover, garden leaf lettuce, onion plants, soybean, and sweet clover. Castor-oil plants and ripe tomato gave the lowest egg records with an average of 18 and 19 eggs, respectively, per female.

9. The average number of eggs produced per female on the control mixed diet was 91.1. Five of the separate foods (wild lettuce, alfalfa, red clover, garden leaf lettuce, and onion plants) exceeded the control egg record. Wild lettuce and alfalfa averaged more than 1.5 times the control average.

10. High reproductive potentials with alfalfa, red clover, and some other cultivated plants lend support to suggestions by other authors that certain farm crops, notably alfalfa, sometimes may be factorial in offering an exceptional nutritional environment for building up higher populations than would occur in similar localities with only natural wild food plants available.

11. No regular agreement appears between egg production and those food preferences listed in other publications. For example, corn silk, reported to be severely attacked in the field, also was eagerly consumed by caged 'hoppers, but egg production of the 'hoppers caged with corn silk alone ranked seventeenth among the 30 foods. The most striking food selection noted in the present experiments was exceptionally marked preference for red clover blossoms. This feeding habit would be very disastrous in clover fields left for seed production, even when only moderate populations of grasshoppers were present.

12. Not all 30 fresh foods were tested during each of the three seasons. However, 12 foods were tested each summer, and a general agreement in results from year to year is evident.

13. Although higher egg production usually is associated with longer adult survival, some noteworthy exceptions were found. Females feeding either on corn silk or cottonwood leaves alone had average survival figures of 27.1 days from the time of initial oviposition until death. This average was the highest among the 30 groups tested on fresh foods. But the average numbers of eggs per female in these two cases were seventeenth and twenty-first in rank. The shortest survival was 9.6 days on mulberry leaves, but the average egg production on this food ranked twenty-fifth.

14. There is a general positive correlation between high average egg production, high average number of pods, and high average eggs per pod.

15. The necessity of abundant succulent food is demonstrated in the preliminary trials with drying foods. While fresh alfalfa gave an average of 140 eggs per female, drying alfalfa produced an average of only 29 eggs. When water was constantly available to 'hoppers on brittle-dry alfalfa, the average egg production came up to 38 per female. Surprisingly good survival records resulted on dry alfalfa plus water, and on drying corn in which stalk tissues furnished some moisture long after the leaves were brittle-dry. Drying alfalfa and drying timothy, however, were not able to support life for very long.

16. Grasshoppers on dry foods became very nervous and consequently more susceptible to slight stimuli which provoked unusually strong escape reactions. It is conjecturally suggested that such hyperirritability may, perhaps, be a factor in the initiation of mass flights which solitary grasshoppers of the American plains make when their food supply becomes somewhat dry and their internal body temperature rises during periods of drouth and high temperature.

17. In cages with dry food and no water, 59.3 per cent of the dead 'hoppers showed some degree of cannibalistic attack, even when dead specimens were removed every morning and noon. No cannibalism occurred in cages with dried alfalfa and water. Only a few cases of cannibalism were noted among the more than 3,000 specimens on succulent food.

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PYTHIUM ROOT NECROSIS OF OATS[1]

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Oats in the seedling and boot stage often are yellow and stunted, and the lower leaves may be brown and dead. These symptoms occur to some extent each year and may be observed in oat fields throughout the state. At heading time the yellow, stunted oat plants tend to regain their normal green color and seemingly recover from the diseased seedling condition. Growers long have recognized that this yellowed and stunted condition of oats during the early spring resulted in a reduction of the oat crop.

Several years' observations indicate that even though the above-described symptoms occur each year, they are most pronounced when conditions are unfavorable for the rapid growth of the oat plant. The yellowing and stunting of oats in April and May has been thought to be a result of one or more conditions, such as cold weather, excess moisture, deficient nitrogen, lack of aeration, root parasites, etc., but few data have been obtained supporting any one of these theories.

In 1922 Gram and Rostrup (2) briefly reported that a root blight caused by *Pythium debaryanum* Hesse and species of Fusarium had been found in some oat and barley fields in Denmark. A species of Pythium parasitic on oat roots was reported by Subramanian (11), Robertson (9), Vanterpool and Truscott (14), Brandenburg (1), and Ho and Melhus (3). None of these investigators, however, studied the interaction of the host and causal organism or the effect of environmental conditions on the activity of Pythium on the roots of oat plants.

A detailed study was undertaken in the spring of 1938 to determine whether the roots of field-grown oat plants were parasitized, and if so, how extensively and by what pathogen or pathogens. The preliminary results of this investigation indicated that species of Pythium were parasitizing the roots and might be partially responsible for the foliage symptoms so evident during April and May. A study of the host-parasite relationship and the effect of the pathogen on the growth and development of the plant itself then seemed necessary.

SYMPTOMS

On oats, Pythium is strictly a seed and root parasite, causing a stunted and yellowed condition of infected plants. Symptoms on the

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glumes, the endosperm, and the developing embryo of germinating seed and on primary and secondary roots are caused by the direct action of the pathogen.

1. *On seed and on young seedlings prior to emergence:* If conditions following sowing are such that the seed requires 1 week or longer to germinate[3], the seed may be rotted and the stand reduced. The glumes of rotted seed are blackish brown in color but remain intact and retain

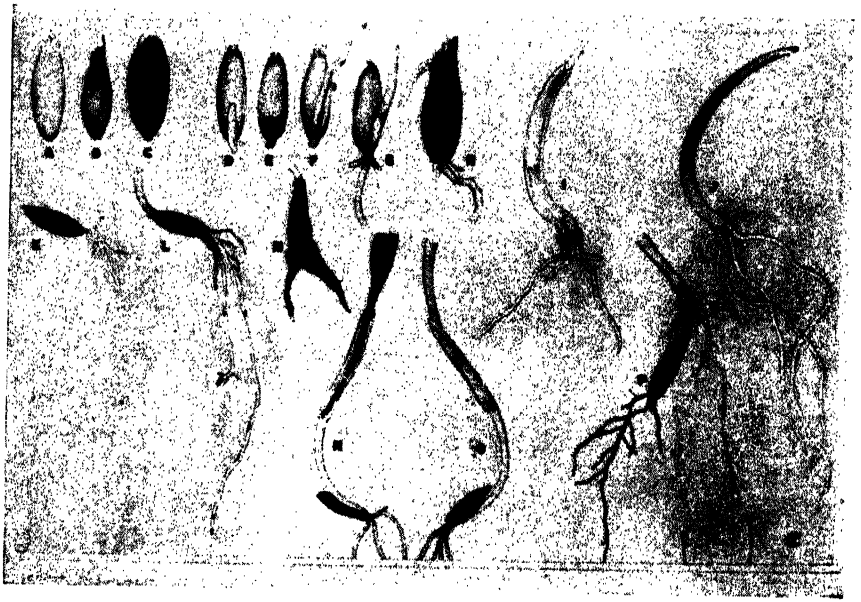


Fig. 1. Symptoms caused by *Pythium debaryanum* on oats. A, unplanted oat seed. B, a seed planted in steamed soil. C, seed planted in Pythium-infested soil. D, normal seed, glumes removed, showing white color of uninfected embryonic tissues. E, seed with discolored and dead embryo. F, G, H, I, young seedlings infected with Pythium; glumes removed except in H. J, K, normal seedlings showing no infection. L, Pythium-infected seedling showing the early stages of root infection. M, infected seedling showing adherence of soil particles to infected parts of the roots. N, O, seedlings showing Pythium lesions on the coleoptile. P, Pythium-infected seedling showing late stages of primary root necrosis and infected subcrownal internode. (Drawn by Marie A. Lincoln).

their original shape as shown in Figure 1,C. If the infected glumes are removed from the caryopsis and the embryo examined, it may show that the embryo was attacked and killed before any apparent development had taken place (Fig. 1,E). The developing seedling often is killed between the time the plumule and the radicle begin to develop and the time the coleoptile appears above the surface of the soil (Fig. 1, F, G, H, I). During this time the organisms may attack the developing radicle before the pri-

[3] The author considers germination to be complete when radicle growth has become evident; following this, the developing embryo is considered a seedling.

mary roots are visible and kill them before they have reached a length of ½ cm. As the roots are killed, the organism grows up into the vascular plate and may grow into the coleoptile and its enclosed leaves. Infected parts usually become brown in color and distorted in shape. Note difference in color and extent of root system of healthy seedling, Figure 1, J, as compared with Figure 1, I.

2. *On coleoptile and subcrownal internode.* Pythium infection on the developing coleoptile causes the appearance of typical blackish-brown lesions. Such lesions are shown in Figure 1, N, O. The lesions are small, circular, dark-brown spots that seldom exceed 2 mm. in diameter. In some cases, however, long, linear, dark-brown lesions that may be ½ to 1 inch in length are found. This type of lesion apparently is caused by the elongation of a coleoptile on which a circular lesion has developed prior to elongation. Both types of lesions usually are restricted to the surface of the coleoptile, because the pathogen rarely penetrates the coleoptile tissues to attack the leaves and stem enclosed within. After the coleoptile emerges and the first leaf is formed, coleoptile lesions may be found above the soil surface.

If infection occurs at the time radicle growth has been initiated, the pathogen may grow upward into the subcrownal internode. If such an infected seedling emerges, the subcrownal internode may reach a length of 1 inch or more, depending upon the depth the seeds are planted in the soil. The entire subcrownal internode, extending from the primary roots to the crownal node, may be infected and killed (Fig. 1, P). Internal spread of Pythium from the point of infection seldom has been observed above the crownal node. In a few cases, Pythium has been found at the base of the stem immediately above the soil surface. In such instances the base of the stem may become discolored and shriveled.

3. *On primary and secondary roots.* Under conditions favorable for *P. debaryanum* the primary roots may be infected as soon as they emerge from the germinating seed (Fig. 1, G, H). The first symptoms on an infected root is a water-soaked, translucent area (Fig. 1, L). These infected primary roots later become reddish-brown throughout, slightly enlarged, and irregular in shape. Young branch roots may be attacked and killed just as they emerge through the epidermal layers of the small primary root (Fig. 1, H). Sometimes soil particles are matted and stuck to the surface of the infected areas by the mycelium of the parasite. The adherence of soil particles to the infected roots, as shown in Figure 1, M, is strikingly characteristic of the disease and may be used as a diagnostic symptom when searching for Pythium root injury.

Lesions found on secondary roots are similar to those already described on primary roots but are more limited in extent.

4. *On foliage:* Oat plants growing in Pythium-infested soil may show stunting, yellowing, and dying of the basal leaves. Stunting begins when the plants are in the seedling stage and becomes increasingly pronounced until heading time. After 2 to 3 weeks, the basal leaves of the infected plants gradually become yellow and begin to die from the tips back.

Yellowing and stunting of oats plants was general throughout many fields in 1938. In such fields, all plants seemed to be equally affected and no differences were found between plants growing on high, well-drained land and those growing on low, poorly-drained land. In 1939, however, yellowing and stunting was restricted to spots. These spots varied from 1 to several yards in diameter and usually were confined to low, poorly drained areas. Spots containing yellow, stunted plants were surrounded by normal-appearing green plants, and the region of transition from yellow plants to green plants was distinct. As the season advanced and the plants began to head, the yellow spots disappeared, because the plants tended to regain their normal green color, and heading more or less screened the stunted condition. The development of the stunted plants, however, was much slower than that of the adjacent green plants.

Stunted, yellowed plants tiller from 1 to 2 weeks later than normal plants. Tillers that appear under these conditions usually dry up and die before they complete development. Under field conditions, yellowed plants seldom have more than one culm but may possess one to several partially developed, dead tillers.

EXPERIMENTAL RESULTS

Prevalence and identity of Pythium on roots of oat seedlings: An attempt was made to trace the prevalence of the pathogen in the field during the growing season. In 1938 and 1939 isolations were made from the infected roots of field-grown oat plants at weekly or bi-weekly intervals during the entire growing season.

One-half to 1-inch pieces of an infected root were thoroughly washed and placed beneath 2 per cent agar-agar in a petri dish. The solidified agar was lifted with a specially flattened needle by the Meredith (8) method and the infected root placed beneath it. This method of isolation reduced bacterial contaminations to a minimum.

The early isolations yielded the highest percentages of Pythium isolates (Table 1). The fact that Pythium was practically the only organism isolated during the first week of May in both years suggested that this organism was the initial causal agent of the root injury. As the seasons advanced, however, isolations yielded a lower percentage of Pythium, and on the final date no Pythium isolates were obtained.

Not only did each successive date of isolation yield a decreased percentage of Pythium isolates, but a higher percentage of so-called secondary organisms, such as Fusarium, Helminthosporium, Rhizoctonia, etc., was obtained as the season advanced. Many of these secondary fungi were tested to determine their pathogenicity on oats, but none of them attacked the oat root to any extent at temperatures below 15°C. For the present, therefore, such fungi may be considered as secondary organisms following Pythium infection.

The significant decrease in the prevalence of Pythium (from approximately 90 per cent to 0) in less than 2 months was attributed to the rapid increase in the soil temperature as the season advanced. In May and June

TABLE 1

PREVALENCE OF PYTHIUM AND OTHER FUNGI ON THE ROOTS OF OAT SEEDLINGS UNDER FIELD CONDITIONS DURING 1938 AND 1939

	No. Isolations	No. Isolates Obtained	Percentage				
			Pythium	Fusarium	Helminthosporium	Rhizoctonia	Unidentified
1938							
April 30	27	23	91.3	4.4	0	4.3	0
May 13	61	58	79.3	10.4	0	5.1	5.1
May 21	100	84	48.8	40.5	7.1	0	3.5
June 8	28	24	25.0	50.0	4.1	0	20.8
June 25	33	17	0	41.2	0	0	58.9
1939							
May 1	50	39	84.6	2.6	5.1	0	7.7
May 10	30	30	80.0	3.3	3.3	0	13.2
May 18	30	26	38.4	8.0	4.0	0	50.0
May 24	80	60	23.1	16.6	3.3	10.1	45.0
June 6	30	22	9.1	36.3	13.0	0	41.0
June 16	33	30	16.6	16.6	6.6	6.6	53.3
June 30	40	30	0	33.3	13.3	6.6	46.6

of both years the average soil temperature increased from 13.5° to 25°C. and the available moisture rapidly decreased in both. The data in Table 1 indicate that Pythium flourished, because there was little competition from other organisms and because the growth of the oat root was retarded by the low soil temperature. As the season advanced, soil temperatures increased, other organisms became active, and Pythium was confronted with strong, rapidly growing, competing organisms. This fact, accompanied by the rapidly changing soil environment, may explain the sudden disappearance of Pythium from the infected oat roots. The increase of Pythium from 9.1 per cent on June 6, 1939, to 16.6 per cent on June 16 was tentatively explained by the fact that 2.8 inches of rain fell between those two dates and lowered the temperature of the soil. By June 30, 1939, Pythium had disappeared from the oat roots, as it had in 1938 at the same date.

During 3 successive years of isolation studies, practically all the Pythium isolates obtained had spherical sporangia. All isolates having oospores with smooth walls were identified as *P. debaryanum*, and those having oospores with spiny walls were identified as *P. irregulare* (7). Frequently isolates were obtained that produced spherical sporangia like *P. debaryanum*, but these strains failed to produce oospores. Occasionally a *P. graminicola* isolate, with its typically filamentous sporangia, was obtained. This occasional occurrence of *P. graminicola* is remarkable, in view of the fact that barley (5), wheat (14), and corn (4) are seriously attacked by species of Pythium with filamentous sporangia. Strains of *P. graminicola* that can parasitize oat roots may exist, but to date none has been found in these studies involving many isolates of Pythium.

Of the species of Pythium commonly isolated from oats, *P. debaryanum* Hesse was more common than *P. irregulare* Buis. *Pythium irregulare,* however, was the most common organism found in the light sandy soil in the vicinity of Conesville, Iowa. Both species, nevertheless, were isolated from oats in widely separated parts of Iowa.

Early greenhouse tests, in which each of the two species was thoroughly tested under controlled conditions, showed that *P. debaryanum* isolates were the most pathogenic and isolates of *P. irregulare* least patho-

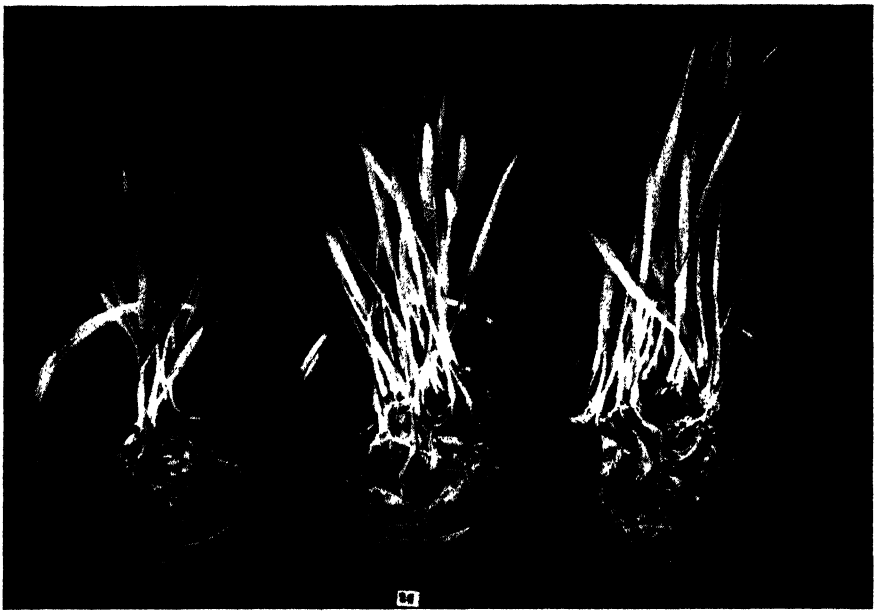


Fig. 2. The total number of oat seedlings grown from 30 seeds of Swedish Select planted in soil infested with (from left to right) *P. debaryanum*, *P. irregulare*, and Noninfested.

genic. Figure 2 shows the relative pathogenicity of *P. debaryanum* and *P. irregulare* on variety Swedish Select. Because of its pathogenicity, *P. debaryanum* was used in all subsequent greenhouse studies.

FACTORS AFFECTING THE PATHOGENICITY OF PYTHIUM ON THE OAT PLANT

Considerable variation in the reaction of duplicate plantings of the same variety of oats in artificially infested soil was noted in preliminary tests. It was evident that a set of standard conditions must be established before the relative resistance of a large number of varieties to *P. debaryanum* could be determined. To do this, a brief study was made of certain factors that could be controlled and which were considered important in determining the severity of Pythium root necrosis in the greenhouse.

Period of development of the organism in artificially infested soil: Since pathogenicity tests were made by artificially infesting steamed soil with cultures of the organism grown on agar-agar, it was desirable to know whether the period of development following the infestation of the soil influenced the host-parasite relationship. To determine this, four 5-inch pots were infested with equal amounts of *P. debaryanum* and incubated 192, 120, 72, 48, 24, and 0 hours before the seeds were planted. Thirty seeds of the variety Bond were planted per pot and the pots were held at 12°–22°C. for 2 weeks. The experiment was repeated twice.

Since the primary roots emerging from the seed were extremely susceptible to Pythium, and many were killed before they reached the length of ½ inch, the severity of the root necrosis was measured by counting the mean number of primary roots that exceeded 1 inch in length in each pot. In general, there were no significant differences in the severity of root necrosis in the six periods of incubation.

The above method of measuring varietal response was used rather extensively during the early part of the investigations. It soon became obvious, however, that primary roots could be attacked at any stage in their development and that root growth was a better measurement for root injury than the number of primary roots that exceeded 1 inch in length. Measurements of root growth, supplemented by stand count and top growth measurements, were accepted as the best criteria for evaluating varietal response to *P. debaryanum* and were used in all further investigations.

Significant differences (Table 2) were obtained in the root growth of plants receiving certain of nine different quantities of inoculum. The mean root growth was 11.4 cm. when one unit of inoculum was placed at the seed level. A unit of inoculum was a pie-shaped segment of agar one-fourth the size of a petri dish. Greater amounts of inoculum significantly increased the amount of root necrosis when compared with one piece of inoculum per pot. Inoculum in excess of one unit, placed at the seed level, was considered too severe for testing varietal resistance. The root injury of diseased seedlings collected from fields heavily infested with Pythium indicated that field infestation did not exceed that produced by placing one unit of inoculum in a 4-inch pot of steamed soil.

Heavy dosages of inoculum increased the amount of injury but also increased the variation between replications. This type of variation was characteristic of greenhouse experiments conducted during the investigations. In practically every variety that was tested in infested soil, certain plants escaped serious injury and appeared normal and healthy. Such plants caused wide variation among replications. In the most susceptible varieties some plants escaped serious injury regardless of how seriously the other plants were parasitized. The presence of these disease-escaping plants could not be explained on the basis of varietal susceptibility.

The response of oat plants grown from primary and secondary seeds: When the panicle of an oat plant matures, one, two, or three seeds (the caryopsis with its adherent lemma and palea) may be formed in each

TABLE 2

STAND, MEAN ROOT GROWTH AND MEAN TOP GROWTH IN CENTIMETERS OF VICTORIA OATS GROWN FROM 30 SEEDS IN 4-INCH POTS RECEIVING 9 DIFFERENT AMOUNTS OF INOCULUM

Amount of Inoculum	Stand			Mean Root Growth			Mean Top Growth			Mean of Three Replications		
	Replication			Replication			Replication					
	I	II	III	I	II	III	I	II	III	Stand	Root Growth	Top Growth
None	26	24	26	11.2	15.1	12.3	9.9	12.4	11.3	25	12.9	11.2
One piece 1 cm. square at seed level	17	20	23	9.0	12.8	11.8	8.4	12.2	11.3	20	11.2	10.6
One unit* at seed level	3	3	5	9.8	12.4	11.9	9.7	11.5	8.6	4	11.4	9.9
Four units at seed level	1	0	2	0.5	0.0	8.3	6.3	0.0	8.9	1	2.9	5.1
One piece 1 cm. square at seed level and one two inches below	19	19	20	4.8	2.2	5.7	7.2	5.1	7.9	19	4.2	6.7
One unit at seed level and one two inches below	7	6	7	0.2	5.3	7.7	2.7	7.7	8.0	7	4.4	6.1
Four units at seed level and four two inches below	2	1	3	1.0	11.0	7.8	4.5	9.5	8.8	2	6.6	7.6
Four units mixed throughout the pot	5	5	1	4.1	5.0	1.5	6.3	6.4	8.5	4	3.5	7.1
Eight units mixed throughout the pot	2	2	0	2.0	3.0	0.0	5.2	9.2	0.0	1	1.7	4.3

* A unit of inoculum as used throughout this paper is equivalent to a pie-shaped segment of agar one-fourth the size of a petri dish.

spikelet (10). These seeds usually are referred to as primary, secondary, and tertiary, and the number developed is determined principally by the conditions under which the plant was grown. The primary seeds are the largest and under adverse growing conditions may be the only ones produced. Secondary and tertiary seeds are smaller than the primary seeds. Under Iowa conditions tertiary seeds seldom are formed.

Primary and secondary seeds of the varieties, Anthony and Swedish Select, were planted in infested soil. The plants were grown for 14 days at 18°–22°C. At the end of the growing period the soil was washed from the roots, and mean root growth was recorded. The experiment was repeated four times.

In general, the plants grown from primary seeds were as seriously attacked by Pythium as plants grown from secondary seeds. The differences that were observed were attributed to vigor and not to a difference in susceptibility between the two groups.

When primary and secondary seeds were planted in steamed, non-infested soil, there were no significant differences in the growth and development of the plants derived from the two groups. In the absence of *P. debaryanum,* one group grew as rapidly as the other, but when the organism was present the primary seeds tended to produce the better plants.

THE EFFECT OF TEMPERATURE ON THE GERMINATION OF OAT SEED SOWN IN PYTHIUM-INFESTED SOIL

In Iowa, oats are sown as soon as the soil is tillable in the spring, usually about April 1. Following sowing, soil conditions may remain so unfavorable for growth that the seed require 2 weeks or longer to germinate. In such cases germination may be so poor that replanting is necessary. Because of poor stands, and because it is known that Pythium may parasitize the seed at the date of planting, an attempt was made to establish the relationship between temperature and the germination of oat seed planted in Pythium-infested soil.

In 1938 the oat variety, Swedish Select, was grown in constant temperature chambers held at 8°, 15°, 20°, 25°, 30°, and 35°C. The seed was sown in 4-inch pots of soil that had been steamed 3 hours at 15 pounds pressure. One series of pots contained soil that had been infested with *P. debaryanum* grown on potato-dextrose agar, and another series contained non-infested soil. The soil was infested with one unit of the inoculum per pot, thoroughly mixed with the upper 2 inches of soil; then 30 seeds were sown in each pot. Two pots of infested and two pots of non-infested soil were placed at each temperature, and the experiment was repeated four times.

The germination of oat seed at 8°, 15°, 20°, and 25°C. was strikingly lower in Pythium-infested soil than in non-infested soil (Table 3). Above 25°C., however, differences between infested and non-infested soil were not so pronounced as at the lower temperatures, and at 30°C. germination in the infested soil equalled that in non-infested soil. At 35°C., the maxi-

TABLE 3

THE EFFECT OF TEMPERATURE ON THE STAND OF VARIETY SWEDISH SELECT GROWN FROM 30 SEEDS IN SOIL INFESTED WITH *P. debaryanum*

Temperature	No. Trials	Emergence			
		Infested Soil	Mean	Noninfested Soil	Mean
8 ° C.	1	2		29	
	2	3		30	
	3	1		29	
	4	2	2.0	30	29.5
15° C.	1	15		29	
	2	11		30	
	3	18		28	
	4	12	14.0	29	29.0
20° C.	1	13		30	
	2	19		25	
	3	13		29	
	4	20	16.2	30	28.5
25° C.	1	18		25	
	2	19		30	
	3	16		29	
	4	22	18.8	29	28.2
30° C.	1	21		23	
	2	23		18	
	3	23		26	
	4	22	22.2	22	22.2
35° C.	1	0		2	
	2	3		0	
	3	0	1	0	0.7

mum temperature for the germination of oats was approached, because only an occasional seed germinated in either infested or non-infested soil. The data indicate that temperatures of 25°C. and lower may result in reduced germination of oat seed planted in Pythium-infested soil. At temperatures at 25°C. and 30°C. the seed germinated rapidly enough to escape serious Pythium injury. The average soil temperature for April in 1938 and 1939 was 13.5°C. The data in Table 3 indicate that a temperature of 13.5°C. may result in more than a 50 per cent reduction in stand.

The effect of temperature on the growth rate of P. debaryanum: The growth rate for *P. debaryanum* was determined by growing the organism on corn meal agar at 1°, 5°, 10°, 15°, 20°, 25°, 30°, 35°, and 40°C. Ten petri dishes were placed at each of the nine temperatures. At the end of 62 hours the mean radial growth of the mycelium was recorded for each temperature and plotted in Figure 3.

The growth rate indicated that *P. debaryanum* should be a serious pathogen to the roots of oat plants at any temperature the latter can be grown. Root necrosis was most serious, however, between 5° and 20° C.,

whereas injury became less pronounced as the temperatures increased from 20° to 30°C. The optimum temperature for Pythium was between 26° and 28°C., and the growth rate of the organism increased constantly until this optimum was reached. In this range of temperature, however, the oat plant attained a rate of growth that permitted it to withstand at-

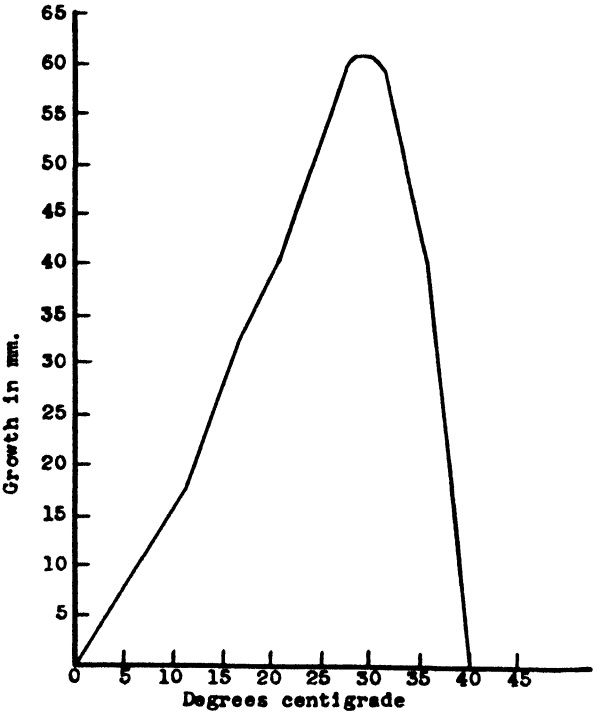


Fig. 3. Growth rate of *Pythium debaryanum* on corn meal agar.

tack. New roots were produced more rapidly than old ones were destroyed, and even though Pythium was quite parasitic at 25°C., it had no serious effect on the growth and development of the plant.

THE PATHOGENICITY OF *P. DEBARYANUM* ON ROOTS OF OAT SEEDLINGS

Table 4 presents the results obtained by growing 32 varieties of oats in duplicate series in Pythium-infested soil. All seeds were 1 year old with the exception of the variety, Anthony, in which case 1-year-old and 4-year-old seeds were planted. The seeds were planted in flats of steamed soil. In one series of flats the soil had been infested with *P. debaryanum* growing on potato-dextrose agar in petri dishes; in another series the soil was noninfested. Each flat was infested with four units of inoculum, which was mixed thoroughly with the soil. Eight varieties of oats were

TABLE 4

THE AVERAGE EFFECT OF *P. debaryanum* ON STAND, ROOT GROWTH AND TOP GROWTH ON 32 VARIETIES OF OATS GROWN FROM 30 SEEDS PER VARIETY IN DUPLICATED SERIES OF FLATS AT 15° C.

Variety	Stand		Root Growth in cm.		No. Secondary Roots		Top Growth in cm.	
	Non-infested Soil	Pythium-infested Soil	Non-infested Soil	Pythium-infested Soil	Non-infested Soil	Pythium-infested Soil	Non-infested Soil	Pythium-infested Soil
C. I. 3601	28	27	4.1	2.5	1.9	1.1	15.7	11.8
Flughafer	29	26	5.9	2.4	2.0	1.7	14.5	11.2
C. I. 3540	29	24	4.2	2.5	1.4	0.4	14.1	10.8
C. I. 3541	30	24	3.9	2.8	1.8	0.7	14.2	11.8
C. I. 3605	29	24	4.4	2.8	1.5	0.8	14.4	11.4
Iogold	30	23	3.7	1.8	1.6	0.4	15.4	12.7
C. I. 3514	29	21	4.7	3.6	1.5	0.5	12.6	12.5
C. I. 3603	27	18	3.7	2.1	1.7	0.6	15.9	10.3
C. I. 3604	30	17	4.2	2.8	2.2	0.8	13.6	10.2
Tama	27	17	3.6	2.6	1.3	0.5	12.1	10.5
Bond	27	17	3.9	3.0	2.6	0.4	15.8	11.5
Victoria	28	17	4.9	2.6	1.4	0.6	18.7	13.1
C. I. 3503	26	16	4.1	3.2	1.8	1.2	12.3	12.8
Swedish Select	29	14	4.2	1.2	1.5	0.8	14.1	8.8
Richland	29	14	4.2	2.0	1.8	1.1	14.4	11.3
Fulghum	28	13	4.3	2.3	1.4	0.0	12.8	9.8
Red Rustproof	30	13	5.3	2.1	1.9	0.3	14.3	10.0
D-67	28	13	4.1	1.7	1.3	0.7	13.9	9.7
Columbia	29	13	4.3	1.2	1.7	0.1	15.5	8.9
Boone	24	12	4.6	1.5	1.6	0.4	14.9	9.3
C. I. 3543	27	12	3.6	3.4	2.1	0.6	12.3	11.8
Landhafer	27	11	5.4	2.0	2.4	0.0	18.3	14.2
C. I. 3500	24	9	3.7	2.4	1.4	0.1	12.6	9.8
Mutica Ukraina	29	8	4.3	1.7	2.0	0.0	18.2	9.6
Markton	27	8	5.6	2.5	2.0	0.2	17.3	11.4
Hancock	25	7	3.5	2.7	1.3	0.0	11.5	8.7
Gopher	29	7	3.5	3.5	1.2	0.3	13.3	11.6
Anthony (1938)	29	6	5.0	2.1	2.3	0.1	16.8	10.2
Marion	25	6	4.8	2.0	1.1	0.0	14.4	7.8
Silvermine	29	2	4.4	1.4	1.9	0.0	16.8	7.2
Nakota	24	1	4.5	1.6	1.9	0.0	15.0	11.2
Anthony (1934)	16	1	3.9	1.5	1.0	0.0	11.7	9.4
Mean	27.3	13.7	4.3	2.3	1.7	0.5	14.6	10.7

sown per flat, with 30 seeds per variety and each variety duplicated in separate flats. The flats were placed in the greenhouse, and the temperature was held at 15°C. to approximately duplicate the average soil temperature for the month of May, 1938, which was 13.5°C. The plants were grown from December 23, 1938, to February 4, 1939.

Pythium tended to reduce germination, to decrease root growth and top growth, and to retard the development of secondary roots. The data are typical of those obtained later in testing many varieties of winter,

[4] These varieties were kindly supplied by Dr. H. C. Murphy, pathologist, Iowa Agr. Exp. Sta. and USDA.

spring, and wild species of Avena[4] in search of resistant varieties. No marked resistance to Pythium injury occurred in the 32 varieties tested. There seemed to be a marked difference in varietal susceptibility, but each of the 32 varieties listed was seriously injured by *P. debaryanum* in the greenhouse test. Figure 4 illustrates the reaction of Flughafer, C. I. 3514 and Mutica Ukraina to *P. debaryanum* at 15°C. The four plants shown in Figure 5 were typical of severely diseased seedlings of highly susceptible varieties.

To determine whether the rate of growth of the oat seedling had any effect on the ability of Pythium to parasitize the oat roots, the 32 varieties

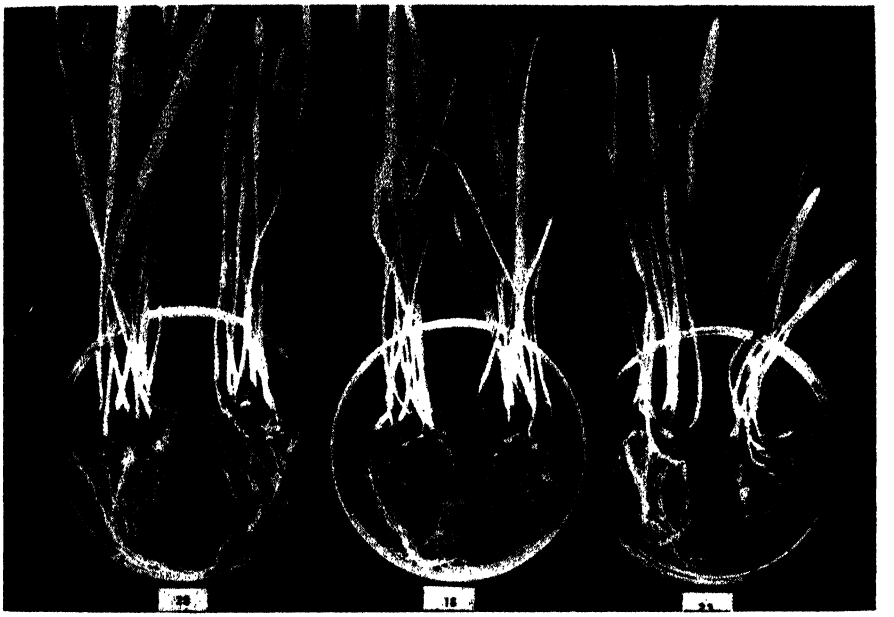


Fig. 4. Varietal response to *P. debaryanum* of (from left to right) Flughafer (28), C. I. 3514, (16) and Mutica Ukraina (23). Ten noninfected plants on left and 10 infected plants on right of each dish. Notice the resistance of Flughafer as compared with Mutica Ukraina.

listed in Table 4 were grown at 21°C. The results (Table 5) indicated that the injury was not so marked at 21°C. as at 15°C. At the higher temperature, differences in stand and top growth of plants grown in infested and noninfested soil were small. At the same time, differences in root growth in infested and noninfested soil were as marked as they were at 15°C. These results indicated that Pythium was a serious parasite at 21°C., but at this higher temperature foliage symptoms were less pronounced. The suppression of foliage symptoms at 21°C. over those at 15°C. appeared to have resulted from a more rapid production of new roots at the higher temperature and not from any reduction in the parasitic abilities of Pythium. At 21°C. environmental conditions apparently were so favorable to the plant that it produced new roots faster than



Fig. 5. Four Swedish Select seedling severely diseased by *P. debaryanum*. Notice that all primary roots were malformed, enlarged and dead. Young secondary roots were also severely injured.

Pythium destroyed them, and pronounced foliage symptoms were not expressed. At 15°C., however, environmental conditions were less favorable for the growth of the host; root destruction surpassed root formation; and pronounced symptoms of Pythium injury developed.

Striking differences were found in the effect of Pythium on 1-year-old and on 4-year-old seed of the variety, Anthony. Ungerminated seed and seedlings of the 4-year-old seed seemed to be more subject to Pythium injury than did 1-year-old seed. At 21°C. 10 per cent of the 4-year-old seed planted in Pythium-infested soil germinated, as compared with 80 per cent germination of the 1-year-old seed. The development of infected seedlings from 4-year-old seed was greatly reduced as compared with the development of infected seedlings from 1-year-old seed. It cannot be said that the 1-year-old Anthony seed was more resistant to Pythium infection than the 4-year-old seed. The differences observed can be at-

tributed to the fact that the 1-year-old seed probably germinated more rapidly, had greater viability and was able to escape invasion and parasitic development of Pythium in seedling roots to a greater extent than the

TABLE 5

THE AVERAGE EFFECT OF *P. debaryanum* ON STAND, ROOT GROWTH AND TOP GROWTH ON 32 VARIETIES OF OATS GROWN FROM 30 SEEDS PER VARIETY IN DUPLICATED SERIES OF FLATS AT 21° C.

Variety	Stand		Root Growth in cm.		Top Growth in cm.	
	Non-infested Soil	Pythium-infested Soil	Non-infested Soil	Pythium-infested Soil	Non-infested Soil	Pythium-infested Soil
Columbia	29	29	4.5	2.4	16.3	13.7
C. I. 3541	30	28	4.4	3.4	12.4	11.5
C. I. 3540	30	28	4.5	2.7	13.6	12.1
Flughafer	30	28	5.3	4.0	14.2	13.9
C. I. 3603	28	28	3.4	2.1	13.5	13.0
C. I. 3543	29	28	4.3	2.3	14.5	12.7
Tama	30	28	3.7	1.8	12.9	8.6
C. I. 3601	29	27	4.8	2.6	12.6	14.4
C. I. 3514	29	27	4.7	2.3	15.1	13.0
C. I. 3503	28	27	4.4	2.2	13.5	12.4
Iogold	29	26	3.3	0.9	13.5	10.3
D-67	30	26	4.3	0.9	12.2	8.3
Richland	30	26	4.6	2.0	13.4	11.9
Mutica Ukraina	28	26	5.2	1.6	15.2	12.2
C. I. 3604	30	25	4.0	2.6	12.2	12.1
Gopher	30	25	5.0	2.0	12.3	11.3
Fulghum	30	25	3.1	2.6	15.5	10.7
Landhafer	29	25	5.8	2.8	18.2	15.7
Bond	28	24	4.4	2.2	15.5	11.2
Anthony (1938)	28	24	4.9	3.2	13.9	11.3
Boone	25	24	3.3	2.0	14.1	12.2
C. I. 3605	30	23	3.7	2.0	13.7	11.8
Markton	28	23	4.9	2.8	16.1	11.2
Red Rustproof	29	23	4.9	3.0	15.6	13.8
C. I. 3500	25	22	4.9	2.2	13.2	10.8
Victoria	30	21	3.8	2.8	16.1	11.2
Marion	27	20	3.5	1.8	13.4	10.4
Swedish Select	29	20	3.5	1.7	12.7	10.8
Hancock	25	18	3.9	0.8	13.1	9.1
Silvermine	29	15	4.1	1.2	13.0	10.2
Nakota	23	9	4.3	2.5	14.0	8.0
Anthony (1934)	18	3	5.0	1.3	6.1	4.8
Mean	28.2	23.4	4.3	2.2	13.9	11.4

4-year-old seed. A similar influence of age of seed on severity of root necrosis was observed in the varieties, Bond and Victoria.

REACTION OF 115 VARIETIES OF OATS TO *P. DEBARYANUM*

When *P. debaryanum* was found to cause a serious root necrosis of oats, a large number of varieties[5] of winter, spring, and wild oats were tested in an attempt to find resistant varieties that could be used for

[5] The varieties were obtained through the courtesy of Dr. T. R. Stanton, senior agronomist, Bureau of Plant Industry, Soils, and Agricultural Engineering, and Dr. H. C. Murphy, pathologist, Iowa Agricultural Experiment Station and USDA.

breeding purposes. The varieties used in experiments previous to this time had failed to exhibit marked resistance to Pythium.

The 115 varieties represented lines developed from six species of wild oats. Twenty-four of the varieties belonged to the *Avena byzantina* C. Koch group, 70 to the *A. sativa* L. group, 16 to the *A. orientalis* Schreb. group, 4 to the *A. nuda* L. group, and 1 to the *A. nudibrevis* Vav. group.

One hundred and fifteen varieties of oats were grown for 14 days in the greenhouse at 15°–20°C. Thirty seeds of each variety were planted in duplicate pots of steamed, non-infested soil and steamed soil infested with one unit of inoculum grown on agar-agar. At the end of the 14-day growing period the plants were removed from the pots and the soil was washed carefully from the roots. Mean stand, root and top growths were recorded in Table 6. The experiment was started March 3, 1940.

An analysis of variance, based on mean root growth indicated highly significant differences in the relative susceptibility of individual varieties. No striking resistance was observed. Variety Coast Black had a mean root growth of 11.2 cm. in infested soil, which was the longest. This was the most resistant variety grown. In the infested series, however, stand was reduced by eight plants, and root and top growths were reduced 2.8 and 1.4 cm., respectively, in comparison with the check. Coast Black showed definite root lesions. Figure 6 shows the Pythium injury to a typically susceptible variety.

The variety, Coast Black, most resistant of the 115 varieties to Pythium, was selected for comparison with other varieties. The least difference for significance in mean root growth was 2.5 cm. Since Coast

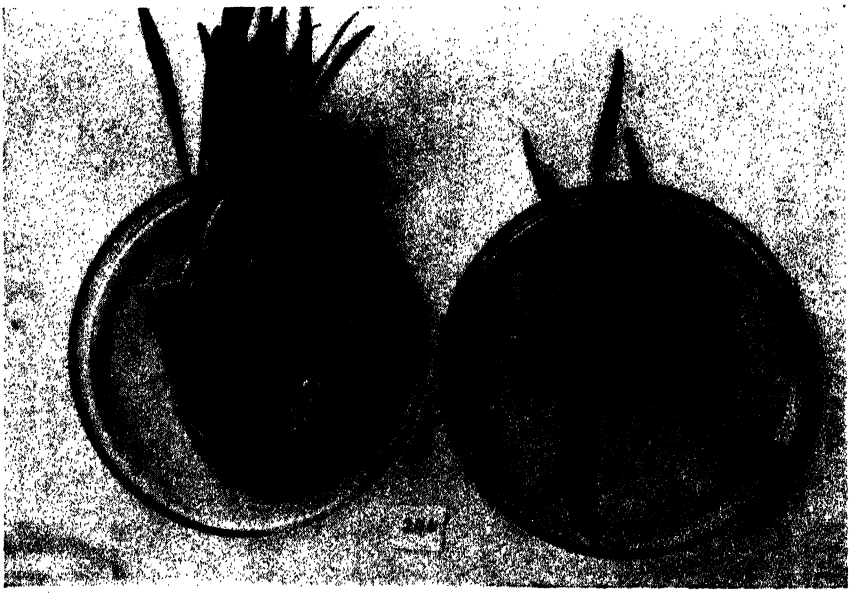


Fig. 6. Pythium injury to Hay oats. Infected plants on right.

TABLE 6

MEAN STAND, ROOT GROWTH AND TOP GROWTH IN CENTIMETERS OF 115 VARIETIES OF OATS GROWN FROM 30 SEEDS PER VARIETY IN DUPLICATED 4-INCH POTS IN THE GREENHOUSE AT 15°–20° C.

C. I. No.	Variety	Noninfested			Infested		
			Mean			Mean	
		Stand	Root Growth	Top Growth	Stand	Root Growth	Top Growth
		Avena byzantina					
1025	Coast Black	29	14.0	9.3	21	11.2	7.9
3215	Black Algerian	25	15.1	9.4	17	10.6	7.4
2911	Cassel	29	13.2	7.7	25	7.8	6.1
2912	Fulmer	29	11.5	7.0	27	8.6	6.5
2823	Early Red Rustproof	30	14.7	8.6	25	10.2	7.2
994	Calcutta	25	12.2	8.5	21	7.2	7.4
1039	Red Rustproof	28	12.5	7.9	22	8.7	8.2
840	Red Algerian	30	13.7	8.9	26	9.6	7.8
3253	Fulgrain	29	12.9	8.3	24	7.1	6.5
2723	Bond	30	13.1	8.2	28	7.7	7.9
2760	Belar	29	13.1	9.4	25	8.9	8.3
966	Navarro	30	12.8	7.7	19	6.9	6.5
2137	Segetal	26	8.0	8.3	14	5.5	4.8
1776	Trisperma	30	11.8	7.1	14	3.7	4.6
3217	Culred	29	15.3	7.9	25	8.8	5.7
2676	Awnless Culred	27	11.6	7.9	11	1.7	4.9
2886	Burt (Nebr. 518)	30	10.7	7.9	18	5.2	5.1
2054	Brunker	29	12.8	8.9	21	8.1	6.6
708	Fulghum	28	12.2	7.3	24	7.8	6.8
2692	Franklin	30	14.3	8.5	22	6.1	6.3
2401	Victoria	30	14.2	8.4	22	9.0	6.8
2820	Columbia	28	15.2	8.8	23	6.1	7.0
2025	Ruakura	28	14.4	8.7	24	9.5	7.5
1799	Sunrise	29	13.6	9.3	26	10.2	7.4
		A. sativa					
480	Roswell	30	8.9	6.5	25	7.4	5.0
606	Almeria	30	12.2	7.6	26	8.7	6.7
838	Hatchett	30	12.7	7.1	16	5.7	4.7
947	Tech	29	9.7	6.2	25	7.1	4.8
1570	Winter Tuff	28	11.4	8.1	25	5.8	6.0
2042	Lee	30	13.3	7.2	27	7.9	5.8
3218	Bicknell	30	14.0	7.4	20	6.1	5.7
273	Culberson	30	12.8	6.5	25	9.0	5.8
2505	Hairy Culberson	26	14.1	6.1	18	8.3	5.9
748	Dwarf	30	13.4	6.6	14	6.6	5.5
1074	Great Mogul	29	13.9	7.1	25	6.9	6.2
1767	Black Bell I	29	11.2	7.4	28	4.9	6.2
803	Victor	29	11.3	8.6	23	4.5	6.5
1877	Black Mesdag	29	13.1	8.4	24	7.2	7.1
1876	Monarch	29	14.0	7.7	23	5.7	6.1
1880	Joanette	28	15.7	8.9	25	6.4	6.9
1092	Early Joanette	30	11.9	8.0	25	5.9	7.0
1878	Black Diamond	29	12.5	8.5	22	4.4	5.2
1879	Awnless Monarch	30	12.8	8.0	18	5.7	6.4
1756	Old Island Black	28	12.9	9.0	20	6.0	6.4
1882	North Finnish	30	12.0	7.9	21	5.4	6.3
1622	Hay	29	10.3	7.3	9	3.7	5.7
2765	Capa	28	12.4	8.2	19	5.0	6.0
1842	Cornellian	29	13.0	8.1	22	6.1	6.2

TABLE 6—(*continued*)

C. I. No.	Variety	Noninfested Mean Stand	Noninfested Mean Root Growth	Noninfested Mean Top Growth	Infested Mean Stand	Infested Mean Root Growth	Infested Mean Top Growth
1692	White Maine	28	12.6	8.1	18	4.4	5.7
602	Terry	28	10.3	7.4	20	4.2	7.7
787	Richland	30	11.7	8.7	14	5.3	6.6
2329	Iogold	30	12.5	8.8	13	3.6	6.5
459	Kherson	30	12.4	8.3	17	3.3	7.1
1154	State Pride	30	13.2	9.7	17	3.9	5.6
2053	Markton	30	10.8	8.4	17	5.3	5.2
603	Madrid	27	13.4	9.5	18	4.7	6.7
831	Aurora	28	12.7	8.5	9	5.2	5.8
1890	Golden Rain	26	13.7	8.1	13	5.1	6.3
1889	Japan	28	12.5	7.0	15	5.3	7.0
1285	Minora	29	14.1	9.1	18	4.2	6.7
1978	Green Russian	30	12.9	9.5	9	4.6	7.1
1888	Awnless Probsteier	27	12.2	9.1	11	1.4	6.4
1656	Probsteier	29	14.3	10.3	11	5.9	6.5
1777	Tabor	29	11.6	9.3	7	2.5	6.3
834	Cole	28	14.7	10.1	10	6.1	7.3
847	Iowar	30	15.4	10.0	18	9.4	7.6
729	Albion	30	11.6	9.1	17	6.7	7.0
2027	Gopher	30	12.9	9.3	11	6.5	7.2
1623	Early Champion	29	13.3	10.2	18	5.4	5.8
1621	Daubeney	30	13.5	9.8	19	8.5	7.9
498	Yakustsk	30	11.6	9.4	16	4.9	6.6
1906	Hudson	29	12.8	9.6	10	5.5	5.8
1685	White Bonanaza	29	13.5	10.0	19	8.5	8.5
1625	Canadian	28	12.9	9.7	20	10.2	9.3
1927	Castleton	27	10.1	7.6	23	7.7	7.9
1624	Early Mountain	30	10.4	8.8	19	5.9	7.9
1709	Tabalsk	29	11.9	8.8	17	4.8	5.7
1894	Clinton	26	11.4	8.5	15	2.3	5.1
846	C. A. C. 72	30	13.6	8.6	17	5.8	6.5
2476	C. A. C. 144	29	13.7	9.4	17	5.9	6.2
1699	Scottish Chief	29	12.1	7.8	13	5.2	6.3
2994	Irish Victor	29	13.9	8.7	14	3.0	5.4
1898	Gothland	30	13.4	8.6	18	5.6	7.2
1684	Danish Island	30	11.6	8.9	21	7.6	8.4
1630	Belyak	29	7.1	8.5	15	5.7	6.5
1375	Swedish Select	25	12.1	8.4	15	5.4	6.3
1262	Lincoln	28	12.1	8.8	20	7.4	7.9
1013	Silvermine	29	12.6	8.8	12	6.1	5.6
2143	Anthony	25	12.2	8.1	18	9.4	8.0
1145	Victory	29	12.1	9.0	9	6.1	6.2
2567	Wayne	30	12.3	8.0	20	5.9	6.3
2783	Sandy	29	9.9	8.5	14	5.4	6.6
1613	Garton 473	30	12.8	8.2	20	8.0	7.2
1884	Garton No. 5	27	10.8	9.6	15	3.0	6.9

Black had a mean root growth of 11.2 cm., all the varieties that had a mean root growth of 8.7 cm. or over were as resistant as Coast Black variety. The interval 11.2 to 8.7 included the following varieties: Black Algerian, Early Red Rustproof, Red Rustproof, Red Algerian, Belar, Culred, Victoria, Ruakura, Sunrise, Almeria, Culberson, Iowar, Canadian, and An-

TABLE 6—(*continued*)

C. I. No.	Variety	Noninfested Mean Stand	Noninfested Mean Root Growth	Noninfested Mean Top Growth	Infested Mean Stand	Infested Mean Root Growth	Infested Mean Top Growth
	A. sativa (*A. orientalis* Schreb.)						
1598	Oriental	30	10.5	8.5	25	6.4	7.2
1862	Garton No. 748	30	10.3	8.9	23	5.5	7.2
1863	Garton No. 784	29	11.2	7.8	21	7.3	7.2
807	Black Rival	30	11.3	9.1	17	5.1	6.8
991	Black Tartar	30	11.2	8.3	26	4.5	7.8
1864	Garton Grey	30	13.5	8.7	19	8.4	7.9
1609	Scizure	28	11.0	7.9	18	4.5	6.4
1606	Golden Giant	28	6.7	7.3	18	4.1	6.1
1612	Garton Yellow	27	11.6	8.5	17	6.2	6.6
1604	Sparrowbill	28	10.1	7.5	22	6.0	6.1
1602	Storm King	26	11.0	8.8	12	2.0	6.0
1599	Tartar King	30	9.4	7.8	14	3.8	6.8
1999	Marvellous	28	10.3	9.0	21	6.5	7.5
2895	Shoemaker No. 7	30	9.2	8.7	21	5.3	7.2
1892	Green Mountain	28	11.6	9.8	18	4.9	7.4
1614	White Tartar	28	11.1	8.7	20	7.3	7.6
	A. nuda						
1966	Fowlide	24	11.6	8.4	3	3.2	2.8
845	Liberty	11	8.4	6.7	0	0.0	0.0
1003	Chinese	18	9.8	7.8	1	3.5	1.5
1770	Mongolian	24	9.8	8.9	4	1.8	1.7
	A. nudibrevis						
2465	Vavilov	20	7.6	6.1	2	1.5	4.8

Least difference for significance = 2.5 cm. for mean root growth in the infested series.

thony. Of these 14 varieties, 9 belonged to *Avena byzantina* and 5 belonged to *A. sativa*.

REACTION OF SIX SPECIES OF WILD OATS TO *P. DEBARYANUM*

Six species of wild oats were grown and tested as described in the above experiment. Two selections of *Avena abyssinica* and three selections of *A. ludoviciana* were included. The haploid number of chromosomes in *Avena nudibrevis* and *A. wiestii*, 7; in *A. barbata* and *A. abyssinica*, 14; and in *A. ludoviciana* and *A. sterilis*, 21. The reactions are presented in Table 7.

An analysis of variance showed highly significant differences in species reaction to Pythium. Each species was attacked by the organism, but *A. abyssinica*, *A. ludoviciana*, and *A. sterilis* were somewhat resistant. In general, both wild species and cultivated varieties that belonged to the 21-chromosome group tended to be more resistant than members of the

14- or 7-chromosome groups. This tendency was very pronounced in the 115 varieties listed in Table 6, which shows that the five most resistant varieties belonged to 21-chromosome species; four of them were in the *A. byzantina* group.

DECREASED YIELD IN VARIETY SWEDISH SELECT CAUSED BY *P. DEBARYANUM*

To obtain some idea of the ability of *Pythium debaryanum* to decrease yield, the variety Swedish Select was grown in 1-gallon stone jars, and the soil moisture was held at 53 per cent of the water-holding capacity or below. Each jar contained an equal amount of steamed greenhouse soil, which had a water-holding capacity of 42 per cent and a field capacity of 22 per cent (dry weight basis). In four jars the soil was infested with one unit of inoculum per jar and was mulched with ½ inch of ground cork to reduce evaporation. Two pots of uninfested soil were held as checks. Fifteen seeds were sown per jar, but each jar was thinned to the first five plants to emerge. In three of the infested jars only three seedlings were produced. The jars were weighed daily, and sufficient water was added to bring the moisture content back to 22 per cent or to 53 per cent of the water-holding capacity. The plants were grown in soil in which moisture never exceeded the field percentage.

The experiment was started on January 10, 1938, and the temperature of the greenhouse was held at 15°C. until the first week in March. By this time it was impossible to maintain a 15°C. temperature throughout the day because of higher air temperatures. Fortunately, these temperature conditions approached those found in the field, *i.e.*, the seed was planted in cold soil, but the temperature increased as the growing season advanced.

TABLE 7

RELATIVE RESISTANCE OF SIX SPECIES OF WILD OATS REPRESENTING THE GROUPS HAVING 7, 14, AND 21 PAIRS OF CHROMOSOMES

C. I. No.	Variety	Noninfested			Infested		
			Mean			Mean	
		Stand	Root Growth	Top Growth	Stand	Root Growth	Top Growth
2456	*A. nudibrevis*	28	8.9	5.6	19	5.5	3.8
2467	*A. barbata*	30	8.7	6.4	21	4.9	4.5
2108	*A. abyssinica*	30	9.9	7.7	26	7.5	6.3
2519	*A. abyssinica*	30	9.1	7.1	30	8.5	6.3
1994	*A. wiestii*	17	9.7	3.7	7	4.4	2.5
2321	*A. ludoviciana*	27	12.0	6.7	21	9.3	6.3
2050	*A. ludoviciana* Dorain	14	9.2	4.5	14	8.6	5.3
1781	*A. ludoviciana* of Ethiopia	30	12.0	6.0	22	10.2	5.4
2723	*A. sterilis* (Alger)	29	14.5	9.8	23	12.4	6.9

Least difference for significance = 3.0 cm. (root).

TABLE 8

EFFECT OF *P. debaryanum* ON THE YIELD AND DRY WEIGHTS OF VARIETY SWEDISH SELECT

Treatment	Stand	Mean Height, Inches				Mean No. Tillers				Mean Dry Weights in Grams			
		April 24	May 7	May 23	June 25	April 24	May 7	May 23	June 25	per Plant	Roots per Plant	Tops per Plant	Seeds per Plant
P. debaryanum	3	14.5	19.8	26.5	27.5	2.0	3.0	4.5	4.5	2.10	0.30	1.8	28.3
" "	5	16.1	24.1	36.0	37.8	2.0	3.0	3.6	3.6	5.10	0.46	4.64	44.2
" "	3	16.7	23.0	28.3	30.8	1.0	2.7	3.7	3.7	5.19	0.49	4.70	44.0
" "	3	13.1	19.7	26.5	28.3	2.0	3.3	5.0	5.0	4.85	0.40	4.45	34.7
Check	5	19.5	28.5	35.2	38.1	3.0	4.8	4.8	4.8	9.50	1.13	8.37	83.4
Check	5	19.5	28.7	38.4	38.6	3.8	4.4	4.4	4.4	8.70	0.83	7.88	82.8

A comparison of height and degree of tillering in infested and in non-infested soil showed that the check plants tended to have better rates of growth (Table 8). In general, the infected plants were 2 weeks behind the noninfected in relation to top growth and the production of tillers. Figure 7 shows the difference that existed between infected and non-infected plants at maturity.



Fig. 7. The effect of *P. debaryanum* on the growth, development and yield of variety Swedish Select. The left pot contains plants growing in noninfected soil.

When mature, the plants were washed from the jars and dried at 100°C. The dry weights obtained showed that the average development of the noninfected plants was approximately twice that of infected plants regardless of whether total dry weights, total root weights, or total top weights per plant were compared. Differences in yield were correspondingly as great, since the check plants yielded 82.8 to 83.4 seeds per plant. Such differences indicate that under certain environmental conditions

TABLE 9

OCCURRENCE OF PYTHIUM IN GREEN AND YELLOWED PLANTS IN 1938 AND 1939

Date	Foliage Color	No. Isolations	No. Isolates	No. Pythium	No. Fusarium Helminthosporium Rhizoctonia Unidentified	Percentage	
						Pythium	Fusarium Helminthosporium Rhizoctonia Unidentified
5/27/38	Green	50	34	20	14	58.8	41.2
	Yellow	50	50	21	29	40.2	59.8
5/28/39	Green	40	32	10	22	31.2	68.9
	Yellow	40	28	4	24	14.2	85.9

1938 chi-square = 2.3; 1939 chi-square = 2.4.

Pythium may be a very important factor in determining the development and yield of the oat plant.

OCCURRENCE OF PYTHIUM IN THE ROOTS OF OAT PLANTS IN GREEN AND YELLOWED AREAS IN THE FIELD

The green and yellow spotted condition of oat fields in Iowa, referred to earlier in this paper, becomes evident during the latter part of May. In 1938, 1939, and 1940 yellow areas began to appear in oat fields throughout the state about May 15, May 19, and May 26, respectively. During each season the spotted condition became progressively more pronounced until the plants began to head. At heading time the yellowed plants regained their green color and could be located only by their reduced size.

In 1938 and 1939 isolations were made from roots of green and yellowed plants found in the same field in an effort to determine whether the fungus flora of yellowed plants differed from that of normal-appearing, green plants. Table 9 presents data typical of those obtained from several fields.

The green plants yielded the higher percentage of Pythium isolates. The chi-square values indicated that there was no significant difference between the Pythium populations of the yellowed and green plants, but in 1938 and 1939 such data were collected repeatedly from fields of oats when yellowing was most pronounced.

In 1940 an attempt was made to determine the difference in size and in tillering in green and yellowed plants. An oat field which exhibited the most pronounced green and yellow areas observed since this study was undertaken was selected near Conesville, Iowa. On May 30 and June 18 plants were collected at random from green and yellow areas throughout the field. The data obtained are presented in Table 10.

On May 30 the green plants were approximately three times as tall as the yellowed and had produced an average of 7 tillers per plant as compared with none for the yellowed plants. By June 18 the yellowed plants were regaining their normal green color, and the green plants were only twice as large. At this time the average number of living tillers on the green plants had been reduced from 7 to 4. This indicated that the green plants, as well as the yellowed, possessed diseased root systems which made it impossible for the plants to mature normally.

One isolation was made from the most severely lesioned root found on each of the green and yellowed plants.

The data in Table 1 show that Pythium is the initial parasite on oat plants in the field. On May 30, 1940, the yellowed plants were beginning to regain their normal green color incident to elongation associated with heading. When isolations were made at the time yellowing first appeared, the green and the yellowed plants yielded approximately the same number of Pythium isolates and other organisms (Table 11). On the other hand, when isolations were made from plants in the same area at the time the yellow, stunted plants were beginning to elongate and assume a richer

TABLE 10

COMPARISON OF SIZE AND TILLERING OF GREEN AND YELLOWED PLANTS COLLECTED FROM THE SAME FIELD IN 1940

Plants Collected May 30				Plants Collected June 18			
Green		Yellowed		Green		Yellowed	
Height in inches	No. tillers	Height in inches	No. tillers	Height in inches	No. tillers	Height in inches	No. tillers
29	9	12	0	36	4	22	0
30	9	12	0	33	6	25	0
23	4	10	0	37	5	24	0
25	6	10	0	35	4	24	2
28	7	9	0	33	3	17	1
31	8	8	0	38	5	18	0
24	5	9	0	40	5	18	0
28	6	12	0	34	3	19	0
30	7	8	0	38	4	18	0
32	7	11	0	35	3	20	0
29	6	13	0	36	4	20	0
28	8	9	0	31	3	19	0
Average:							
28	7	10	0	36	4	20	0

green color, the highest percentage of Pythium isolates was found in the green plants. In fact, attempts to isolate Pythium from stunted plants during the last week of June have been futile. From June 20 until the end of the growing season Pythium has been isolated only from plants that did not show yellowing, and even then it was difficult to isolate.

NUTRIENT DEFICIENCIES IN RELATION TO THE ROOT NECROSIS OF OATS

It has been shown that none of the varieties which were tested was free from Pythium injury. Experiments dealing with the temperature relationships between Pythium and the oat plant, the relationships between the age of seed and the severity of the root necrosis, etc., indicated that any factor which retarded the growth of the plant permitted the organism to become more of a limiting factor in that growth. By associating these facts with the yellow, stunted conditions that appeared in the field each year, an attempt was made to determine whether mineral deficiencies had any influence on the expression of foliage symptoms or on the severity of the root necrosis. Related experiments were performed under the controlled greenhouse conditions and in the field.

The effect of nutrient deficiencies on infected and noninfected plants grown in quartz sand: The varieties, Victoria and Bond, susceptible to Pythium root necrosis, were grown in pure quartz sand in the greenhouse. The sand was washed with a 10 per cent solution of HCl, thoroughly washed in tap and distilled water, steamed for 4 hours at 15 pounds pressure, and placed in steamed 5-inch pots in the greenhouse.

TABLE 11

THE OCCURRENCE OF PYTHIUM IN PLANTS STARTING TO TURN YELLOW AND IN PLANTS RECOVERING FROM THE YELLOWED CONDITION

Date	Foliage Color	No. Isolations	No. Isolates	No. Pythium	No. Fusarium Rhizoctonia Unidentified	Percentage	
						Pythium	Fusarium Rhizoctonia Unidentified
5/30/40	Turning yellow.	24	24	24		100	
	Green.....	24	21	21		100	
6/18/40	Yellow (regaining green color).....	24	24	2	22	8.3	91.7
	Green.....	24	23	6	17	26.1	73.9

June 18, 1940, chi-square = 2.6.

Six pots of each variety, containing 30 seeds per pot, were subjected to eight nutrient solutions. When the seeds were planted, 3 of the 6 pots receiving any one solution were artificially infested with one unit of inoculum. The remaining 3 pots were noninfested and were held as checks. Equal amounts of the respective nutrient solutions were applied each day to the designated pots. Every 7 days 125 cc. of distilled water were added to all pots to prevent the accumulation of salts. The distilled water leached through the quartz sand rapidly, and the nutrient solution applications were continued 8 to 10 hours later. Victoria was planted February 2, 1942, and Bond, February 10, 1942. The varieties were grown 38 and 28 days, respectively, at 18° to 22°C. When deficiency symptoms appeared in the check pots, the plants were examined, and dry weights were recorded in Table 12. Eight nutrient solutions and a check (distilled water) were used. These solutions are enumerated in the table.

Pythium root necrosis was very severe in the infested pots regardless of the nutrient deficiencies. In the series using Victoria in Pythium-infested soil, deficiencies of magnesium, nitrogen, potassium, and phosphorus-potassium gave significantly poorer root growth than the complete nutrient solution. No significant differences in the effects of the nutrient solutions were obtained on the root weights of Victoria in the noninfested soil or on Bond in either the infested or noninfested soil. In these three instances, variation within replications was greater than the variation caused by the nutrient solutions.

In the Victoria check series, plants developed an excellent root growth in each of the nutrient solutions as shown in Figures 8, 9, and 10. Top growth, however, in the N, PN, and KN-deficient pots was greatly reduced. In each of these cases, top growth was yellow, stunted, and the leaves were dying from the tip ends. The N deficiency, however, had not affected root growth at the time the plants were dug (Fig. 10).

The Victoria plants in the infested K-deficient series showed a typical vein clearing. Plants in the noninfested K-deficient series appeared normal and healthy. In pots in the noninfested series receiving distilled water, the plants were stunted, yellow, and had many dead or dying leaves. The roots of these same plants made a remarkable growth, as shown in Figure 9, which indicated that the oat plant could survive for 38 days or longer under greenhouse conditions with seed reserves as the sole source of nutrient.

Bond plants in the Pythium-infested pots receiving the magnesium, potassium, potassium-nitrogen, potassium-phosphorus-deficient, and complete nutrient solutions were very chlorotic and slightly stunted. The noninfested pots receiving the same solutions were normal green in color and very vigorous. Plants grown in the infested and noninfested sand receiving the nitrogen and phosphorus-nitrogen-deficient solutions and distilled water were chlorotic and stunted. The infested plants, however, were more seriously affected than the noninfested. Plants in both infested and noninfested pots in the phosphorus-deficient series appeared to be normal and healthy.

TABLE 12

MEAN DRY ROOT AND TOP WEIGHTS IN GRAMS PER POT OF VARIETIES BOND AND VICTORIA GROWN IN TRIPLICATE IN PYTHIUM-INFESTED AND NONINFESTED QUARTZ SAND RECEIVING EIGHT DIFFERENT NUTRIENT SOLUTIONS

Composition of Solution	Victoria				Bond			
	Top Weights		Root Weights		Top Weights		Root Weights	
	Ck.	Inf.	Ck.	Inf.	Ck.	Inf.	Ck.	Inf.
Distilled water	0.762	0.325	0.910	0.299	0.933	0.647	1.622	0.516
Complete*	1.448	0.470	1.106	0.242	1.330	1.383	1.078	0.694
" −Mg	1.428	0.400	1.053	0.140	1.412	1.043	1.275	0.763
" −N	0.628	0.239	1.306	0.101	0.917	0.908	1.352	1.022
" −K	1.341	0.331	1.017	0.148	1.215	1.007	1.100	0.653
" −P	1.092	0.334	1.256	0.321	1.360	0.810	1.185	0.953
" −PN	0.806	0.322	0.913	0.298	1.037	0.770	1.385	1.012
" −KN	0.624	0.426	1.153	0.213	1.285	0.885	1.400	0.721
" −PK	1.463	0.286	1.325	0.082	1.340	0.837	1.455	0.657

* The modified Knop's solution with the designated substitutions for missing elements was used as outline by Loomis and Shull (6).
Least difference for significance in Pythium-infested Victoria root weights = 0.139.

The results obtained with Bond and Victoria in the above experiments were similar to those obtained in a preliminary experiment of the same nature. The outstanding observations made in the course of these experiments was that deficiency symptoms were expressed by infected plants several days before they appeared in noninfected plants. In general, the presence of the organism retarded the growth of the plants and eventually caused chlorosis and stunting. A nutrient deficiency produced similar

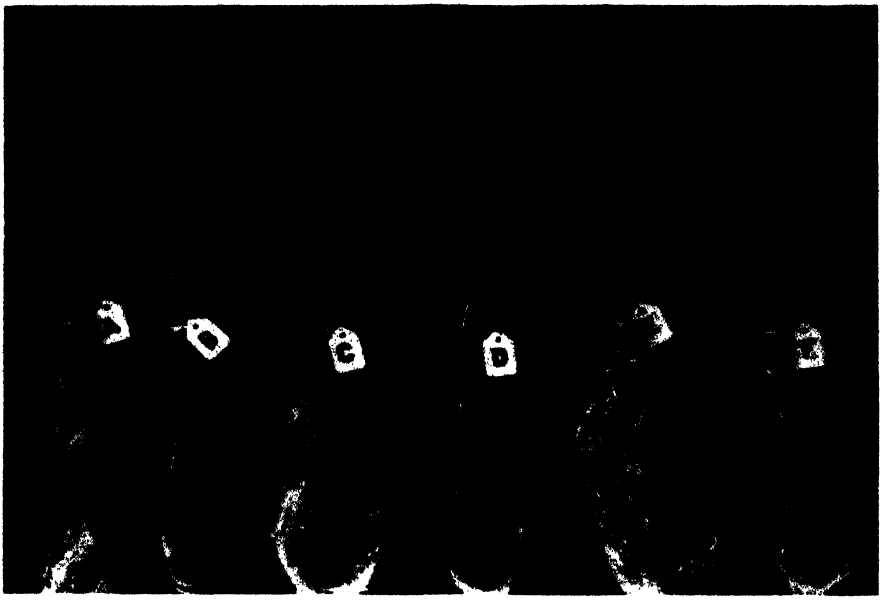


Fig. 8. Oat plants, variety Victoria, grown from planting 30 seeds in Pythium-infested and noninfested quartz sand with Knop's solution.
A. Without K, not infested
B. Without K, infested
C. Without P, not infested
D. Without P, infested
E. Complete Knop's, not infested
F. Complete Knop's, infested

symptoms. When the plant was subjected to the organism and a nutrient deficiency, the growth of the plant was retarded by the accumulative effects of the two factors.

The Victoria plants definitely showed that certain mineral deficiencies aggravated the root necrosis. The same deficiencies on Bond did not affect the seriousness of the root necrosis, probably because the plants were not grown long enough. The fact that any particular deficiency did or did not make the plant more susceptible was of minor importance. The mineral deficiencies retarded the growth of the plant, and this abnormal condition aggravated or increased the injury caused by Pythium. Adversely low temperatures and the planting of old seed have proved to be

just as important as mineral deficiences in producing abnormal plants and in determining the severity of a Pythium attack.

The response of the varieties Boone and Marion, grown in field plots heavily infested with Pythium, to certain fertilizer amendments: The prevalence and wide distribution of *P. debaryanum* and related species of Pythium in oat fields throughout the state and the fact that no variety of oats was highly resistant indicated that Pythium root necrosis would be

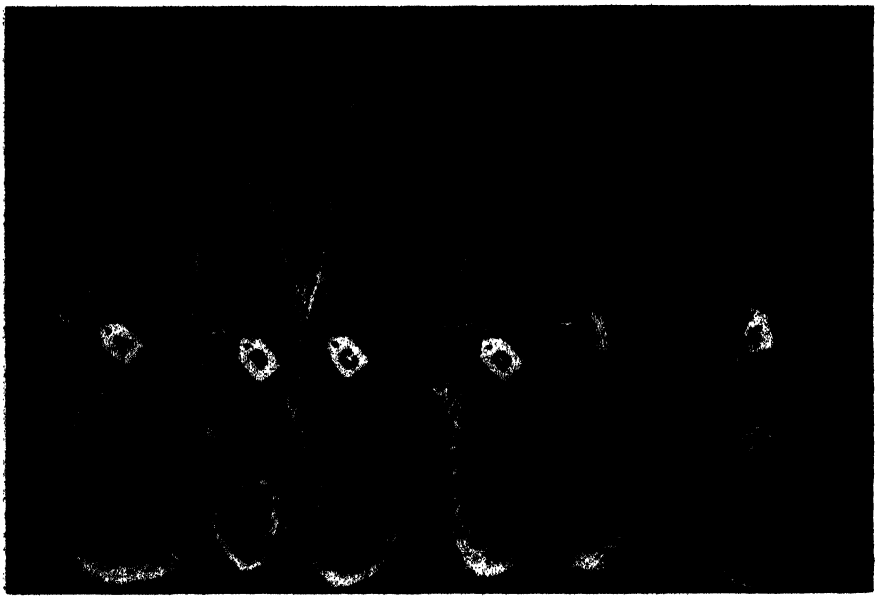


Fig. 9. Oat plants, variety Victoria, grown from planting 30 seeds in Pythium-infested and noninfested quartz sand, with Knop's solution.
A. Without PK, not infested
B. Without PK, infested
C. Without Mg, not infested
D. Without Mg, infested
E. Distilled water, not infested
F. Distilled water, infested

difficult to control. This immediately raised the question whether applications of commercial fertilizers to infested soils would increase or decrease the root necrosis.

Vanterpool (13) working on the browning root rot of wheat caused by *Pythium* spp., found that phosphatic fertilizers and farm manure gave adequate control of such organisms in the infested prairie soils of Canada. He considered that the improvement in growth of infected wheat plants, resulting from these amendments, was due to the production of a large number of rapidly growing roots. The experiments did not indicate that the phosphatic materials increased the wheat plant's resistance to Pythium.

In earlier work Vanterpool (12) analyzed 66 pairs of prairie soil

samples collected from diseased and healthy wheat fields. The chemical analysis of these 66 pairs of soil samples showed that 90 per cent of the diseased areas had less available phosphorus and 74 per cent contained more nitrate nitrogen than the healthy areas. These results indicated that where the P/N ratio was relatively large the wheat plant was not subjected to serious injury. If the P/N ratio was small, the wheat plant was seriously attacked by Pythium. These findings made it desirable to deter-

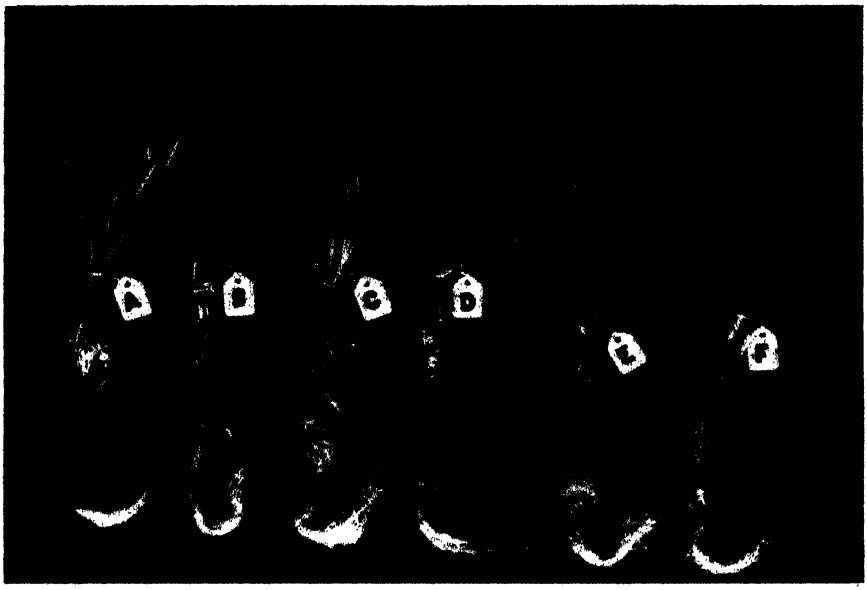


Fig. 10. Oat plants, variety Victoria, grown from planting 30 seeds in Pythium-infested and noninfested quartz sand, with Knop's solution.
A. Without N, not infested
B. Without N, infested
C. Without PN, not infested
D. Without PN, infested
E. Without KN, not infested
F. Without KN, infested

mine whether the same or a similar relationship existed in the root necrosis of oats.

An attempt was made, in 1941, to determine the influence of certain fertilizers on Pythium root necrosis of oats under field conditions. The following fertilizer applications were made on Buchner sand and on Buchner sandy loam: none, phosphorus (500 lb. $CaH_4(PO_4)_2$ per acre), potash (80 lb. KCl per acre), nitrogen (200 lb. $NaNO_3$ per acre), complete fertilizer, 6-8-12, 350 lb. per acre), stable manure (ten tons per acre).

The fertilizers were broadcast, worked into the top 2 inches of soil, and the plots were arranged in a 6 x 6 Latin square on each of the two soil types. Three rows of the two rust and smut-resistant varieties of oats,

Boone and Marion, were planted in rows 8 feet long at 1-foot intervals in each plot. Seven and one-half grams of seed were planted in each row, and the two inside rows of the two varieties were harvested from each of the plots.

The two soil types used for the experiment were selected because both were known to be heavily infested with Pythium. The Buchner sandy loam had a higher level of fertility, a greater water-holding capacity, and a higher organic content than the Buchner sand. The fertilizers were expected to give their most beneficial results when applied to the Buchner sand. Yield data collected from the Buchner sand and Buchner sandy loam plots are recorded in Tables 13 and 14.

The fertilizers failed to give significant differences in yield on either type of soil. Boone significantly outyielded Marion on the Buchner sandy loam and, in general, increased yields due to fertilizers were slightly below the level required for significance. Marion, however, gave a significantly greater yield than Boone on the Buchner sand.

In controlled greenhouse experiments, Boone was somewhat more resistant to Pythium root necrosis than Marion. Since the Buchner sand had a lower water-holding capacity than the heavier Buchner sandy loam, water could become a limiting factor to plant growth in the light sand before it would in the sandy loam. By the time the plants were beginning to head, the light Buchner sand was very dry and hot, and the oat plants were small and stunted. These same conditions reduced the Pythium flora to the extent that they could not be isolated after the first of June. Possibly these conditions made it possible for Marion to outyield Boone on the Buchner sand, because Pythium was not a limiting factor during the last 6 weeks of the growing season.

Plants on the Buchner sandy loam never became stunted throughout the growing season. The black, heavy soil retained its moisture and made it available to the plants for a longer period of time than the Buchner sand. The presence of this moisture tended to reduce the soil temperatures, and Pythium was isolated from the Buchner loam soil 3 weeks later than it could be obtained from Buchner sand. Thus, Pythium was more of a limiting factor in the Buchner loam, and Boone outyielded the more susceptible Marion.

The response of Boone and Marion, grown on Buchner sand treated with chloropicrin, to certain fertilizer amendments: Under greenhouse conditions the exact degree of injury that Pythium caused to the oat plant was determined relatively easily. In the field, this was much more difficult because so many uncontrolled factors existed, such as soil-inhabiting pathogens. To at least partially overcome this difficulty, the soil was treated with chloropicrin.

A plot of Buchner sand was treated with chloropicrin (480 lbs. per acre) in the late fall of 1940. The chloropicrin was applied at a depth of 6–8 inches, and the treated soil was sealed with water for 48 hours following the application. On April 1, 1941, oats were planted in the plot, and the following fertilizer treatments were used: none, phosphorus (500

TABLE 13

YIELD OF GRAIN IN GRAMS PER ROW FROM BOONE AND MARION VARIETIES, GROWING ON BUCHNER SAND WITH FIVE FERTILIZER AMENDMENTS. CONESVILLE, IOWA, 1941

Check		Phosphorus		Potassium		Nitrogen		Complete		Manure	
Boone	Marion	Boone	Marion	Boone	Marion	Boone	Marion	Boone	Marion	Boone	Marion
48	56	58	47	64	54	62	76	61	55	69	79
58	40	63	44	57	46	62	83	50	57	63	60
60	63	74	90	48	38	78	107	78	87	72	61
74	77	66	87	60	76	82	110	49	85	82	77
118	126	62	72	86	130	53	78	54	80	99	84
82	76	66	60	83	100	68	116	60	61	80	100
50	69	86	100	81	93	54	72	79	95	93	104
43	59	80	80	71	80	44	89	83	86	92	92
86	98	76	94	68	55	62	77	73	100	60	70
88	96	72	66	91	82	59	60	80	115	66	58
52	66	70	96	74	70	87	84	92	103	67	73
56	67	62	100	84	40	80	102	64	68	65	60
Var. mean											
64.6	74.4	69.6	78.0	72.2	72.0	65.1	76.2	68.6	82.6	75.7	76.5
Plot mean:											
1668		1771		1731		1815		1815		1826	

TABLE 14

YIELD OF GRAIN IN GRAMS PER ROW FROM BOONE AND MARION VARIETIES GROWN ON BUCHNER SANDY LOAM WITH FIVE FERTILIZER AMENDMENTS. CONESVILLE, IOWA, 1941

Check		Phosphorus		Potassium		Nitrogen		Complete		Manure	
Boone	Marion	Boone	Marion	Boone	Marion	Boone	Marion	Boone	Marion	Boone	Marion
125	192	196	180	191	180	182	118	216	179	222	194
207	207	168	208	224	201	182	142	224	162	195	225
140	160	190	170	256	117	216	173	190	168	143	214
177	175	161	195	206	168	187	175	214	196	224	186
238	216	207	200	188	171	203	182	176	158	206	186
202	186	208	166	177	198	180	215	160	168	234	181
140	148	238	226	233	230	207	196	238	206	179	192
128	147	238	108	248	230	227	192	240	235	187	174
190	173	250	276	249	237	184	112	211	229	214	238
156	212	236	198	167	200	198	127	248	269	180	76
236	194	220	260	211	246	208	228	234	174	117	81
230	160	205	233	226	226	196	230	212	166	117	224
Var. mean: 180.8	180.8	209.8	201.7	214.7	200.3	197.5	174.1	213.6	192.5	184.4	180.9
Plot mean: 2170		2469		2490		2230		2437		2195	

lb. $CaH_4(PO_4)_2$ per acre, potash (80 lb. KCl per acre), nitrogen (200 lb. $NaNO_3$ per acre). The plots were arranged in a Latin square, and varieties Boone and Marion were planted in 8-foot rows, 1 foot apart on every plot. Seven and one-half grams of seed were planted per row. The 6 x 6 Latin square (fertilizer plots) was placed adjacent to the 4 x 4 Latin square (chloropicrin plots) to compare the growth of oat plants in soil to which chloropicrin had not and had been applied, respectively.

The Buchner sand plot treated with chloropicrin gave greatly increased yields over the nontreated Buchner sand plot (Tables 13, 15). The increase in yield was caused by the chloropicrin which partially eliminated the pathogenic organisms from the soil, particularly during the first 3 or 4 weeks of the growing season. During this time it was difficult to isolate Pythium from the chloropicrin plots, but it was isolated at will from the adjacent 6 x 6 Buchner sand plot that had not been treated with chloropicrin. The plants growing in the chloropicrin-treated soil grew rapidly during April, May, and the first week of June, and stunting was not observed until after the sandy soil became hot and dry. Plants on the adjacent 6 x 6 Latin square were badly stunted by the first week in May and were suffering from a continuous root pruning by Pythium. When hot, dry weather came in June and July, the plants on the chloropicrin plots had the most efficient root systems and could obtain more water from the rapidly drying Buchner sand than plants with badly parasitized root systems. This enabled the plants growing in Buchner sand, treated with chloropicrin, to have a longer growing period before water became the limiting factor.

An analysis of variance in the yields obtained on the chloropicrin-treated soil showed that none of the fertilizer applications significantly increased the yields. Marion, however, was a significantly higher yielder than Boone, which was attributed to the relative lack of Pythium in the chloropicrin plot.

TABLE 15

YIELD IN GRAMS PER ROW FROM BOONE AND MARION VARIETIES, GROWN ON BUCHNER SAND TREATED WITH CHLOROPICRIN FOLLOWED BY THREE FERTILIZER APPLICATIONS

Check		Phosphorus		Potash		Nitrogen	
Boone	Marion	Boone	Marion	Boone	Marion	Boone	Marion
184	149	158	157	149	170	140	138
146	141	120	152	170	162	142	145
151	188	146	147	160	174	162	156
143	170	178	190	134	138	163	192
148	143	110	169	156	150	128	157
141	130	132	159	128	148	161	114
110	138	108	126	118	157	129	110
127	80	114	126	114	145	112	129
Var. mean 143.8	142.4	133.3	153.3	141.1	155.5	142.1	142.6
Plot mean 1145		1146		1187		1139	

The influence of certain fertilizers, broadcast and drilled with the seed, on the root necrosis of oats: The failure of the fertilizers used in 1941 to significantly increase the yield indicated either that these fertilizers had been applied in insufficient amounts or that the Buchner sand and sandy loam soils were at a high level of fertility for the oat plant. In 1942 another experiment was conducted using various fertilizers applied at greater rates than in 1941.

Commercial $CaH_4(PO_4)_2$, KCl, $NaNO_3$, complete fertilizer (6-8-12), flowers of sulphur, and Formacide were broadcast and drilled in the respective plots as indicated in Table 16. Broadcast applications were distributed evenly throughout the plot, and the fertilizer was raked into the top 2 inches of soil just before the seed was sown. Drilled applications were evenly distributed in the furrows at the time the seed was sown. The plots were 6′ x 8′, and two rows of Boone and two rows of Marion were planted at 1-foot intervals in each plot. Seven and one-half grams of seed were planted per row. Alleys 2 feet wide were left between every two plots, and the plots were arranged in a randomized block with three replications. Data were collected from the two inside rows of oats planted

TABLE 16

MEAN STAND AND MEAN TOP GROWTH IN INCHES OF 10 PLANTS SELECTED AT RANDOM IN EACH OF THREE REPLICATIONS OF VARIETIES BOONE AND MARION GROWN ON BUCHNER LOAM WITH VARIOUS FERTILIZER AMENDMENTS. CONESVILLE, IOWA, 1942

Fertilizer	Application	Mean Top Growth in Inches		Mean Plot Stand
		Boone	Marion	
800 lbs. $CaH_4(PO_4)_2$	Broadcast	17.3	20.7	460
400 " "	Drilled	17.3	23.7	399
600 " KCL	Broadcast	17.3	21.3	379
300 " "	Drilled	19.0	23.0	232
800 " $NaNO_3$	Broadcast	25.0*	28.7*	432
400 " "	Drilled	24.0*	27.7*	334
800 " 6-8-12	Broadcast	26.0*	28.7*	417
400 " "	Drilled	25.3*	29.3*	336
2000 " Sulphur	Broadcast	21.0	24.7	389
1000 " "	Drilled	19.7	24.0	397
800 " Formacide	Broadcast	17.7	20.3	344
400 " "	Drilled	18.7	22.3	317
400 " $CaH_4(PO_4)_2$	Broadcast	19.3	22.3	340
200 " "	Drilled	19.3	21.0	362
300 " KCL	Broadcast	19.7	22.7	379
150 " "	Drilled	19.7	22.3	353
400 " $NaNO_3$	Broadcast	24.0*	28.3*	411
200 " "	Drilled	24.3*	36.3*	286
400 " 6-8-12	Broadcast	23.0*	24.0	418
200 " "	Drilled	22.7*	26.7*	295
1000 " Sulphur	Broadcast	19.3	20.0	399
500 " "	Drilled	19.3	23.0	357
500 " Formacide	Broadcast	19.0	21.3	410
250 " "	Drilled	21.0	23.3	378
Check		18.0	23.0	387

* Significantly greater than check.

on any one plot. The plots were planted April 3, 1942. Environmental conditions were unusually hot and dry throughout April and the first week in May. The remainder of May and the month of June were abnormally wet and cool.

The seed continued to germinate for 3 weeks after it was planted, and the stand was irregular. During this period of germination, the plots that received $NaNO_3$ and the complete fertilizer did not show foliage symptoms of Pythium root necrosis as did the plots receiving the other fertilizers. By the third week in May these differences were very pronounced. The oats in all the plots were chlorotic and stunted, except for the eight plots that had received $NaNO_3$ and the complete fertilizer. Plants grown in these plots had a deep green color and appeared to be growing normally and vigorously. Mean top growth measurements, made June 1, are recorded in Table 16. The data show that $NaNO_3$ and the complete fertilizer gave highly significant increases in top growth in variety Boone when broadcast at 800 or 400 pounds and drilled at 400 and 200 pounds per acre. Marion also showed significant increases for the same fertilizers except when 400 pounds of complete fertilizer were broadcast. No other fertilizer gave significant increases in top growth over the nontreated plot. Sulphur, however, when drilled at 1,000 pounds and broadcast at 2,000 pounds per acre seemed to cause a slightly better growth of plants in comparison with the nontreated plot. This increase did not reach the level of significance.

The mean plot stand counts for each of the 25 plots tended to be higher when the fertilizers were broadcast, which indicated that much smaller applications should be used when drilled with the seed.

Pythium and other soil-borne organisms were isolated from the roots of plants grown in each plot, and serious root necrosis was observed in each of the plots. In the $NaNO_3$ and the complete fertilizer plots, however, the plants seemed to have more roots and appeared to be capable of producing new roots at a greater rate than the plants obtained from the other plots. No evidence was obtained that indicated that any fertilizer interfered with or stimulated the attack by Pythium. The $NaNO_3$ and the complete fertilizer did not make the plants resistant to attack but made it possible for them to develop new roots rapidly and to continue an apparently normal growth even though the organism was actively parasitizing the roots.

SUMMARY

Pythium debaryanum Hesse caused serious root necrosis to oats grown in Iowa during the years 1938 to 1942, inclusive.

Isolated from field-grown oat plants, *P. debaryanum* was found to be the predominating organism in the infected roots in the early part of the season. Eighty to 90 per cent of the isolations made from field-grown seedlings during the first week in May yielded Pythium. Successive isolations made in late May and in early June gave decreased percentages of Pythium and increased percentages of secondary organisms. Pythium was not isolated after June 25, although many attempts were made in 1938, 1939, and 1940.

Greenhouse studies showed that *P. debaryanum* was capable of causing a serious seed rot and root necrosis at temperatures at and below 25°C., becoming especially serious between 8° and 15°C. The average soil temperature in 1938 and 1939 for the top inch of soil in Iowa during the month of May was approximately 13.5°C.

Greenhouse studies in artificially infested soil showed that temperature, size, and age of seed planted and the application of varying amounts of inoculum were important factors in determining the severity of the Pythium disease.

Two hundred and thirty-two varieties of oats were grown in artificially infested soil, and the varieties Coast Black, Black Algerian, Early Red Rustproof, Red Algerian, Ruakura, and Flughafer were the most resistant varieties in the collection. No marked, outstanding resistance, however, was observed in any of the 232 varieties tested. Commercial varieties and wild species having 21 pairs of chromosomes were more resistant than those with 14 and 7 pairs.

Plants grown in infested soil had a reduced rate of growth and delayed tillering. The oven dry weight of plants grown in infested soil was approximately one-half that of plants grown in noninfested soil. Plants of the variety, Swedish Select, grown to maturity in Pythium-infested soil yielded approximately one-half as much as did plants grown in noninfested soil.

The prevalence of Pythium on the roots of yellow and green plants in the field depended on the time the isolations were made. If isolations were made when yellowing was first observed, the yellow plants tended to give the highest percentage of Pythium isolates. The roots of the yellow plants were rotted more rapidly than roots of green plants, secondary organisms entered rapidly, and Pythium was isolated from green plants at a later date than it was from yellow plants.

Nutritional deficiencies increased the detrimental effects of injury caused to the plant by Pythium root necrosis. Infected plants became chlorotic and stunted several days before noninfected plants when grown under the same nutrient deficiency.

Heavy applications of $NaNO_3$ and a complete fertilizer (6-8-12) prevented field-grown plants from becoming stunted and chlorotic. These applications made it possible for the plants to produce new roots and to replace those badly parasitized by Pythium. Since the rate of root replacement was high, the plants continued to grow vigorously and appeared normal. No evidence was obtained that indicated that $NaNO_3$ and complete fertilizer (6-8-12) increased a plant's resistance; they merely made it possible for the plant to endure the attack.

Throughout the investigations on oats, any factor that retarded the growth of the plant made Pythium injury more pronounced but did not noticeably affect the pathogenicity of the organism. Plants grown under optimum conditions of temperature, moisture, fertility, etc., continued an apparently normal development in the presence or absence of *P. debaryanum*. If, however, any factor became limiting to the optimum development of the plant, Pythium seemingly became more destructive.

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SOME FACTORS DETERMINING THE INFECTION OF CORN BY USTILAGO ZEAE (BECKM.) UNGER[1]

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Corn smut, caused by *Ustilago zeae* (Beckm.) Unger, is one of the serious endemic diseases of the major crop of the Upper Mississippi Valley. The only effective control measure that appears to be feasible at present is the development of resistant varieties. The location of resistant strains for use in a breeding program is hampered by the lack of a reliable and consistent method of producing epiphytotics of corn smut for testing purposes.

The most favorable approach to such a procedure seems to be Davis' spiral whorl technique, which was modified from one of Brefeld's three original methods of inducing smut of corn. The various methods of producing infection which have been used, their effectiveness, and the objections to them have been thoroughly discussed by Davis (6) and Walter (19). The present paper reports further studies designed to improve and elucidate certain details of Davis' technique.

METHODS

The monosporidial cultures used in this investigation were obtained from chlamydospores by the usual isolation technique (6) employing a Chamber's (2) micromanipulator. The isolated sporidia were grown on carrot agar.

A series of collections of smut galls was made from Iowa corn fields during the winter of 1938-39. The cultures obtained were designated by the number of the gall and a letter indicating the order in which the sporidium was isolated from the chlamydospores of that particular gall. Combinations of sporidia were indicated by combinations of the letters of the sporidia of a gall, 10ab, or of the numbers of the cultures from two galls, 10a, 15a.

Infection of the corn plants was induced by Davis' (6) method. A clump of sporidia the size of a pinhead was transferred from a stock culture to each of a series of flasks containing 25 ml. of sterilized carrot decoction (6). Each monosporidial isolate was grown in a separate flask. The cultures were incubated in the laboratory for 7 days, and were shaken vigorously once or twice on or after the fourth day to produce a uniformly turbid growth. On the seventh day, equal parts of two mono-

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sporidial suspensions were mixed together and an equal part of freshly prepared 1 per cent fish-oil soap added. The inoculum so prepared was referred to as the "treated inoculum."

The corn used in these tests was planted thickly and thinned 7 to 10 days before inoculation to well-spaced plants of uniform size. From October 21, 1939, to April 13, 1940, plantings of inbred line Os 426 were made every 2 weeks in greenhouse ground beds. The plants were artificially subjected to infection about 5 weeks after planting when they were 12 to 15 inches tall. The records of gall production were obtained 15 days after inoculation. Nontreated plants were included in all tests to serve as checks. In no case, however, were any of these plants observed to show any infection. To save space the negative reaction of these nontreated plants is not recorded in the results reported.

The results obtained in the experiments are presented in a summarized form consisting of a percentage factor and a severity factor. Thus, 80.7/2.45 indicates that 80.7 per cent of the plants subjected to infection produced galls and on this 80.7 per cent of the plants, the average severity of gall production was 2.45.

The average severity was determined on the basis of a scale developed during the course of the investigation in an attempt to secure a better measure of the pathogenicity from a limited number of plants, and to represent the severity of the disease, as measured by the extent of gall formation on the leaves, on a numerical basis. The scale (see Fig. 1) was based on the percentage of leaf area of the plant hypertrophied, not upon the volume of gall tissue produced. The plants pictured represent the mean of the range of severity represented by each value. The severity rating of a group of plants would vary somewhat in records made by several workers unless they had had previous experience in its use. This variable has been tested by repeating the records made on various experiments and has been found to be small. A more serious discrepancy may have entered as a result of the decision to consider and record all evidences of infection other than gall formation, i. e., yellowing, anthocyan production, necrosis, etc., as a zero rating and, therefore, the same as noninfected plants. This procedure seemed to check with the experience of other workers (1, 6, 9, 14) and to introduce the least difficulty in expressing consistent results.

In certain cases a product of the percentage and severity factors will give a complete picture of the rating of two or more treatments in a test, but in most cases it seems more complete, and the nature of the data and their interpretation is clearer when both figures are recorded. This is clearly indicated in Tables 2, 5, and 7. The latter practice was followed throughout.

EXPERIMENTAL

Surface Tension Depressant: Davis (6) developed a technique of inoculating the corn plant with a sporidial suspension of *Ustilago zeae* by dropping it, after the addition of a surface tension depressant, into the



Fig. 1. The arbitrary scale, based upon the extent of leaf gall formation, employed to give a numerical rating to the severity of disease induced by *Ustilago zeae*.

leaf whorl. Dickson and Bowman (7), using chlamydospores, also found the method successful under field conditions, while Walter (19) found no benefit from using the depressant under greenhouse or field conditions. Presumably the perfect surface tension depressant for such use must possess four qualities. It must (1) be nontoxic to host and pathogen, (2) lower the surface tension of the inoculum sufficiently to enable it to reach the growing point of every plant unless the leaf whorl was blocked, (3) have a margin of safety between injury to the host and maximum surface tension depression, and (4) be of a stable and uniform constitution.

Fish-oil soap, as used by Davis (6), does not satisfactorily fulfill all the above requirements, and in an attempt to find a substitute, triethanolamine oleate, sodium ricinoleate, dreft (sodium salts of sulfonated higher alcohols), saponin, ethylene glycol, kerosene, and numerous oils were tested. Although saponin, sodium ricinoleate, kerosene, and the oils gave low tensiometer readings, they definitely were inferior in greenhouse inoculation trials. Dreft had a mild phytocidal action but showed a high fungicidal action. Attempts to increase infection by using greater concentration of the detergents resulted in greater burning of the plant.

Triethanolamine oleate, showing nearly the same surface tension depression as fish-oil soap when used in carrot decoction, was the more effective when used with the treated inoculum as may be seen in Tables 1 and 2.

The triethanolamine oleate was prepared by mixing, at room temperature, seven parts of oleic acid with one part of 90 per cent triethanolamine in 92 parts of water. It appeared that the volume of oleic acid need not be accurate. The triethanolamine oleate so prepared and diluted with 15 parts of water (0.5 per cent) was equal in action to the fish-oil soap, and when used with 10 parts of water (0.727 per cent) was superior to the fish-oil soap (Table 1).

A comparison was made of the reaction between the inbred line of corn Os 426 and eight fertile combinations of monosporidial cultures of *U. zeae* when fish-oil soap and triethanolamine oleate were used as depressants. The results based on 10 plants per treatment are reported in Table 2. The superiority of triethanolamine oleate over fish-oil soap is again evident. There is an apparent tendency for the extent of the gall produc-

TABLE 1

THE EXTENT OF GALL PRODUCTION INDUCED BY "TREATED INOCULUM" WHEN TRIETHANOLAMINE OLEATE AND FISH-OIL SOAP WERE USED AS SURFACE TENSION DEPRESSANTS

Surface Tension Depressant	Concentration in Percentage	Smut—Percentage Severity*
Triethanolamine oleate	0.727	99.3/6.0
Triethanolamine oleate	0.500	90.9/4.1
Fish-oil soap	0.333	75.9/2.83

* See explanation in "Methods," pg. 401.

TABLE 2

THE EXTENT OF GALL PRODUCTION INDUCED BY VARIOUS FERTILE COMBINATIONS OF *Ustilago zeae* AS INFLUENCED BY THE SURFACE TENSION DEPRESSANT EMPLOYED

Fertile Combination	Treatment		
	Triethanolamine Oleate	Fish-oil Soap	
	10 Plants to Each Treatment	10 Plants to Each Treatment	12 Tests of 10 Plants Each for Each Treatment
11ac	100/6.5	80/3.5	92.5/3.38
10ab	100/6.3	70/4.43	87.5/3.17
50ad	100/6.1	100/3.9	81.6/2.63
33ae	100/6.0	70/3.43	85.0/2.86
81bc	100/5.7	80/2.88	81.7/2.72
49ab	100/5.5	80/3.75	70.0/2.44
93ab	100/3.8	30/2.0	54.2/1.74
8ac	90/3.1	60/2.17	70.8/1.98
Average	98.75/5.4	71.25/3.26	77.9/2.6

tion by the various fertile combinations to shift uniformly to a higher rating when triethanolamine oleate was used, but relative differences in virulence were still evident.

The value of the scale used to measure the extent of gall production over mere percentage of diseased plants was clearly shown when only a few plants were used and the percentage of infection was high. There was a rather close relationship between the severity factor and the percentage factor for gall production when fish-oil soap was used as the detergent, especially when a large number of plants was involved. When triethanolamine oleate was used as the surface tension depressant, penetration of the "treated inoculum" apparently was consistently deep enough that only one plant out of 80 failed to produce galls. The differences in virulence of the various fertile combinations, however, still are evident from the severity factor.

The fourth column in Table 2, which is a summary of 12 trials similar to that recorded in the third column, gives a somewhat truer picture of the relative virulence of the various combinations because of the larger number of plants involved. An arrangement of the fertile combinations according to their relative virulence, as measured by one test of ten plants for each combination with triethanolamine oleate used as the detergent, was quite similar to the summary of 120 plants to which the fish-oil soap-treated inoculum was applied.

No successful surface tension depressant was found that was non-toxic to the host plant. A successful surface tension depressant allowing the sporidia of the smut organism to reach the young growing tissue that is susceptible to infection is likely to injure this same tissue. A scale was developed reading from zero for no visible effect, 1 for yellowing, through various degrees of necrosis to 9 for a plant near death. Using

this scale, readings of the phytocidal action of various detergents were obtained in three different tests. Table 3 indicates that 0.5 per cent triethanolamine oleate is not as severe as fish-oil soap, while 0.727 per cent triethanolamine oleate is more severe.

Dilution of inoculum: The use of a dilute sporidial suspension would greatly facilitate the preparation of the inoculum for large scale inocula-

TABLE 3

THE EXTENT OF NECROSIS INDUCED BY "TREATED INOCULUM" WHEN TRIETHANOLAMINE OLEATE AND FISH-OIL SOAP ARE USED AS SURFACE TENSION DEPRESSANTS

Surface Tension Depressant	Percentage Concentration in Sterile Carrot Decoction	Average Severity of Necrosis
Fish-oil soap	0.333	2.5
Triethanolamine oleate	0.727	3.0
Triethanolamine oleate	0.500	2.1

tions. The results of experiments conducted to test such a dilution of the treated inoculum using various types of detergents are reported in Table 4. Carrot decoction was much more useful as a diluent, allowing a much higher percentage and severity of infection than distilled water. It would appear, therefore, that diluting the culture 1 part to 100 with carrot decoction and using triethanolamine oleate at 0.727 per cent would not greatly affect the value of the inoculum.

ANALYSIS OF CULTURES

A study was made of the virulence of eight fertile combinations of monosporidial cultures on the inbred line of corn Os 426. Ten plants of Os 426 were subjected to infection by each of eight fertile pairs of monosporidial cultures of the "treated inoculum" method in each ground plot

TABLE 4

THE EFFECT OF VARIOUS SURFACE TENSION DEPRESSANTS, DILUENTS, AND DILUTIONS OF INOCULUM ON THE EXTENT OF GALL PRODUCTION INDUCED

Dilution Treatment	Surface Tension Depressant			
	Fish-oil Soap 0.33%	Triethanolamine Oleate 0.72%	Triethanolamine Oleate 0.50%	Hypodermic Injection (checks)
Full strength	90/3.7	100/4.6	92.5/3.5	100/6.0
1/50 dilution with distilled water	12/1.3	88.8/4.2	85.7/3.4	
1/50 dilution with carrot decoction	87.6/3.4	93.3/4.9		
1/100 dilution with distilled water	0.0/0.0	75/2.8		
1/500 dilution with distilled water				100/5.4

in the greenhouse at about 2-week intervals until 12 tests had been completed. The procedures described under "Methods" were followed throughout. The data from these 12 trials are summarized in Table 5. Except for 49ab, all combinations maintained their relative positions in all tests. The advantage of using severity rather than percentage infection is again indicated, as the combinations seem much more stable in this category.

The combination of cultures, 49ab, showed a marked tendency to induce necrosis rather than gall production (Fig. 2). This reaction was more marked in some tests than others, especially in the latter half of the work, suggesting that it might be associated with high temperatures. Christensen and Stakman (5) reported that different collections of *Ustilago zeae* induced different degrees of necrosis, depending upon the line of corn into which they were hypodermically injected. Walter (18) made a cytological study of the infection of corn by sporidia and chlamydospores of *U. zeae* and found that only a few cells were killed at the point of penetration in hypersensitive reactions. The use of a rather concentrated suspension of the pathogen in this work may account for the rather extensive necrosis produced by this line.

REACTION OF THE COMPOSITE INOCULUM

Since *U. zeae* is a species consisting of many races (4, 5, 16, 17), it appears that for large-scale field tests of a number of varieties of corn the inoculum should consist of several races. The greater the number of monosporidial cultures involved, the greater might be the possibility of securing the most virulent combination or race for each of the corn lines tested. Presumably if a composite inoculum consisting of eight fertile pairs of monosporidial cultures was used, there would be a chance for 64 fertile combinations to occur in the plant. Such a composite inoculum was tested in the greenhouse in comparison to each of the eight pairs of cultures composing it, using the "treated inoculum" in each case. The data reported in Table 6 represent the average of nine replications of ten plants each for each inoculum.

The lowered smut reaction with a composite inoculum agrees with the results recently reported by Kernkamp and Martin (10). A possible explanation for the lowered reaction with the composite inoculum is that the mycelia that invade most rapidly are not the ones which stimulate the formation of the largest galls. These results seem to agree, although using the reverse process, with those of McNew (12), who found that the subcultures of a moderately virulent culture of *Phytomonas stewartii* (E.F.S.) Bergey et al., ranged from weakly virulent to very virulent.

SOLOPATHOGENIC CULTURES

Chilton (3), Christensen (4), Eddins (8), Stakman, *et al.* (16, 17) have reported the occurrence of solopathogenic cultures of *U. zeae*. In the present work, out of 125 monosporidial isolates tested, two (51b and

TABLE 5

THE REACTION OF EIGHT FERTILE COMBINATIONS OF *Ustilago zeae* ON THE INBRED LINE OF CORN Os426

Plot No.	11ac	10ab	33ae	81bc	50ad	49ab	8ac	93ab	Ave.
1.....	100/2.70	90/2.78	80/2.50	80/2.63	80/1.38	80/2.13	70/2.14	90/1.78	83.7/ 2.255
2.....	90/3.55	50/2.80	80/2.50	70/2.14	60/2.83	90/2.00	40/1.25	50/1.60	66.25/2.33
3.....	80/3.50	70/4.43	70/3.43	80/2.88	100/3.90	80/3.75	60/2.17	30/2.00	71.25/3.26
4.....	100/3.20	80/1.50	80/3.13	100 2.90	100/2.40	100/2.40	100/2.40	90/2.33	92.5 /2.51
5.....	80/5.13	100/3.20	100/3.70	100/2.10	80/2.87	100/3.30	90/2.11		92.7 /3.20
6.....	100/3.2	100/2.7	100/2.6	100/2.6	80/3.0	60/3.5	80/1.6	20/1.0	80.0 /2.53
7.....	100/4.20	100/3.50	100/3.90	100/4.40	90/2.10	90/1.55	100/2.40	60/1.66	92.5 /2.96
8.....	90/3.22	100/4.30	90/3.90	50/2.60	70/2.57	10/1.00	80/2.38	80/1.14	71.25/2.64
9.....	100/3.1	80/2.25	70/2.0	90/2.9	90/2.55	30/1.0	50/1.25	20/1.0	66.25/2.0
10.....	100/4.0	100/4.2	100/2.0	100/2.9	100/3.9	100/2.4	80/1.625	60/1.5	92.5 /2.81
11.....	90/3.1	90/3.1	50/2.0	60/2.0	70/1.9	50/1.8	50/1.8	30/2.00	61.25/2.21
12.....	80/3.25	90/3.0	100/3.1	50/2.6	60/2.17	60/2.17	50/1.6	30/2.67	65.0 /2.63
Ave.....	92.5 3.38	87.5/3.17	85.0/2.86	81.7/2.72	81.6/2.63	70.0/2.44	70 8/1.98	54.2/1.74	77.9 /2.615



Fig. 2. Characteristic leaf symptoms induced with "treated inoculum." Notice the region of injury at "1" induced by the toxicity of the "treated inoculum."
Leaf A: Symptoms induced with the mating 10ab. Note the restricted gall formation with yellowing and anthocyan production at "2" and the well-developed gall at "3" which is nearer the base of the leaf.
Leaf B: Symptoms induced with the mating 49ab. Note the absence of galls and the necrotic areas in the region of infection comparable to the space between "2" and "3" on leaf A.

TABLE 6

THE REACTION ON INBRED Os426 OF A COMPOSITE INOCULUM AND EIGHT OF THE POSSIBLE SIXTY-FOUR FERTILE COMBINATIONS OF THE SIXTEEN CULTURES COMPOSING IT

Inoculum	Per cent Infection Severity
11ac	91.1/3.63
10ab	92.2/3.41
Composite	89.5/3.48
33ae	86.7/2.96
50ad	82.5/2.77
81bc	81.1/2.78
49ab	64.6/2.33
8ac	71.1/1.88
93ab	41.3/1.62

97e) were solopathogenic. Monosporidial subcultures from these two cultures gave a similar reaction, indicating their true solopathogenicity.

Chlamydospores produced by these solopathogenic cultures were germinated and single sporidia isolated at random. Of the 11 monosporidial isolates from chlamydospores produced by 51b, three isolates were of one sex, three of the other, and five were solopathogenic. Of seven monosporidial isolates from chlamydospores produced by 97e, six were of one sex, one of the other, and none were solopathogenic.

All seven solopathogenic cultures produced a small "pinhead" type of leaf gall which was so different from that induced by the 125 normal heterothallic combinations that it is difficult to compare the two (Fig. 3). Galls induced by the solopathogenic lines at nodes or the union of leaf blade and sheath were normal, large galls. Of the original two solopathogenic lines, 97e was the more virulent on the inbred line of corn Os 426. Galls induced on Golden Bantam sweet corn by solopathogenic isolates, as pictured by Christensen (4), appear quite similar to those induced by normal heterothallic combinations and not at all like the galls induced by the seven lines studied here.

VACUUM INOCULATION

A method which might allow the use of small plants was modified from that used in oat tests by Leukel (11) and on barley and wheat by Moore (13). The "treated inoculum" was dropped into the spiral whorl of potted seedlings in the fourth-leaf stage and the plants then placed under a bell jar which was evacuated to 70 cm. of mercury. The plants were subjected to this reduced pressure for 15 minutes and then returned to the greenhouse bench. The plants had not been watered for 24 hours before treatment but were not wilting.

In five tests, 25 Golden Bantam sweet corn plants were subjected to the vacuum treatment. The 22 plants which became infested had a severity rating of 8.5. Of these plants, 18 were dead within 25 days. Of 25



Fig. 3. A comparison of the leaf galls on Golden Bantam sweet corn induced by a normal pair of haploid cultures (10ab), Plants 1 and 2, with those induced by a solopathogenic culture (5lb), Plants 3 and 4. Note the mild "pinhead" type of gall produced on a leaf blades, but normal gall at the union of the leaf blade and sheath of Plant 4. Inoculation by hypodermic needle.

plants similarly treated but not subjected to the reduced pressure, 9 became infected with a severity of 2.0. No effects were observed on 7 plants subjected to the reduced pressure after treatment with sterilized carrot decoction plus 0.33 per cent fish-oil soap.

SUMMARY

Davis' spiral whorl method of inoculating corn with *Ustilago zeae* (Beckm.) Unger in the greenhouse was improved by using triethanolamine oleate as a detergent. Triethanolamine oleate proved to be the best detergent to use with the sporadia suspended in carrot decoction. The triethanolamine oleate was less toxic to the host tissues, more constant in its composition, and promoted a higher degree of infection of greater severity than fish-oil soap, sodium ricinoleate, dreft, saponin, ethylene glycol, kerosene, or several oils when used as detergents.

The sporidial suspension of *U. zeae*, after being diluted 1 to 100 with carrot decoction, plus triethanolamine oleate, still produced a high degree of infection of great severity.

The range of pathogenicity exhibited by matched pairs of sporidia, when tested on a single inbred line of corn, extended from the production of a few small galls to many large galls or to necrotic areas.

Two solopathogenic lines were isolated that produced pin point leaf galls but normal-sized nodal galls.

A composite inoculum of eight matched pairs of sporidia was less virulent than the most virulent of the matched pairs.

A vacuum method of inoculating corn with *U. zeae* was tested.

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DISTRIBUTION OF EUPHORBIACEAE IN IOWA, WITH SEED KEYS[1]

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A program of making seed keys was initiated by the Iowa State College Seed Laboratory in 1943. The keys are designed to aid the seed analyst as well as the taxonomist in seed and plant identification. In the first paper (10) keys to the seeds of 14 species of the Geraniaceae were presented. In this paper the Euphorbiaceae in Iowa have been chosen as the second study. One key to the genera and three keys to the species have been made.

The family, Euphorbiaceae, contains a number of plants of economic significance. One member, *Euphorbia esula* L.[2] (leafy spurge) is a serious noxious weed. Others, while not noxious, are decidedly weedy; namely, *E. maculata* L.[3] (upright spotted spurge), *E. supina* Raf.[4] (prostrate spotted spurge), and *Acalypha rhomboidea* Raf. (three-seeded mercury). *Euphorbia cyparissias* L. (cypress spurge) and *E. marginata* Pursh (snow-on-the-mountain) were introduced into gardens. They have escaped from cultivation and have become somewhat weedy.

The fruit of the Euphorbiaceae develops from a superior ovary which is three-celled and has central placentation. One or two ovules are borne in each cell and are suspended near the apex of the ovary. The three-lobed dehiscent capsule may be leathery or parchment-like in texture, and the surface may be smooth, wrinkled, pubescent, or strigose. The three-branched style often remains attached to the capsule. Only one seed develops in a locule. In some species not all of the locules produce seed.

The seeds of Euphorbiaceae considered here usually possess a caruncle at the apex, a raphe extending from apex to base, a coat with wrinkled ridges, striations, tubercles, pits or a smooth surface. Internally, these anatropous seeds have a centrally placed straight embryo with large flat cotyledons. The embryo is encircled by an abundant oily endosperm. (Fig. 8.)

The seeds in this study can be separated taxonomically by the following characters: shape, size, and color of the seed; surface of the seed coat; and type of caruncle. The capsule character, when present, is used in the keys as an additional aid in identification.

[1] Journal paper No. J-1263 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 86.

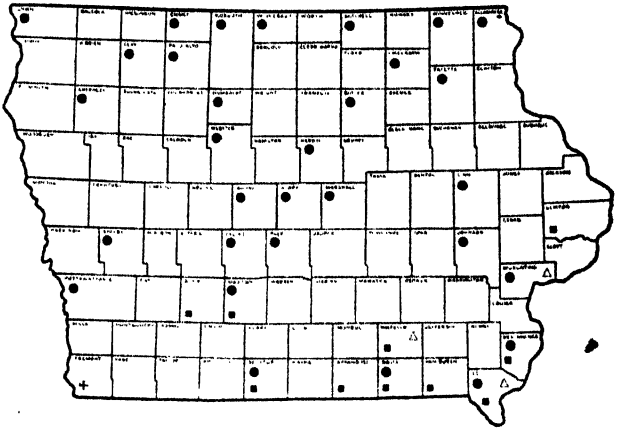
[2] *Euphorbia virgata* Waldst. & Kit. (9). Croizat (2a) considers the phase of "*E. esula*" occurring in North America to be *E. intercedens* Podp.

[3] *Euphorbia preslii* Guss. of recent manuals.

[4] *Euphorbia maculata* L. of recent manuals.

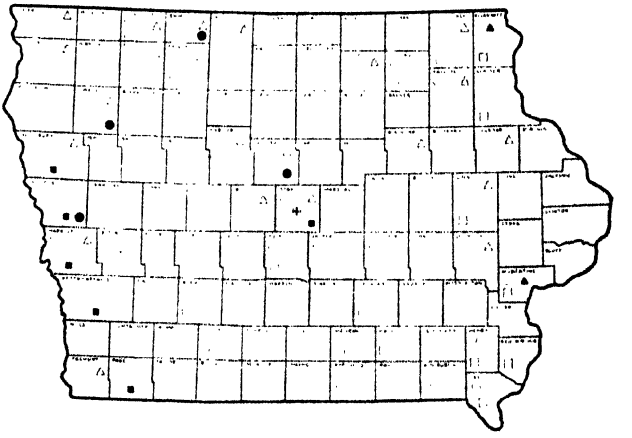
29. Distribution of *Acalypha*

- ● A. rhomboidea
- △ A. gracilens
- ■ A. virginica
- ⊕ A. ostryaefolia



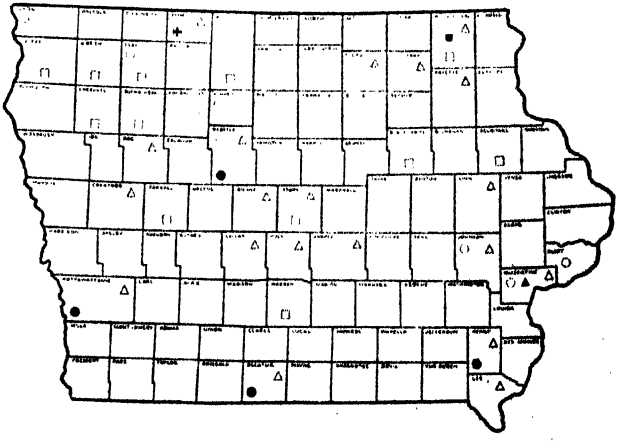
30. Distribution of *Euphorbia* (Chamaesyce)

- ● E. serpyllifolia var. genuina
- ○ E. supina
- ▲ E. geyeri
- △ E. glyptosperma
- ■ E. serpens
- □ E. maculata
- ⊕ E. missourica var. intermedia



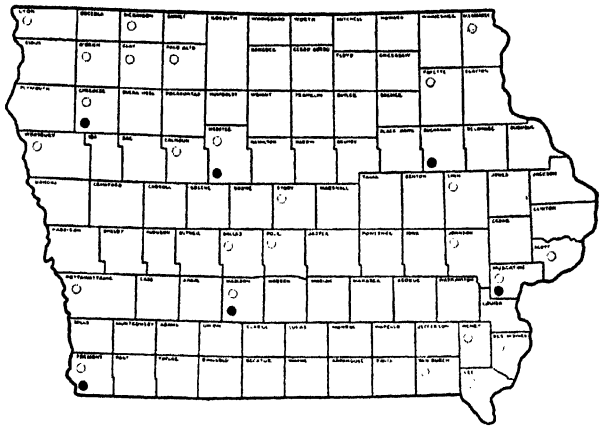
31. Distribution of *Euphorbia* (Tithymalus)

- ● E. obtusata
- ○ E. dictyosperma
- ▲ E. peplus
- △ E. cyparissias
- ■ E. commutata
- □ E. esula
- ⊕ E. platyphylla



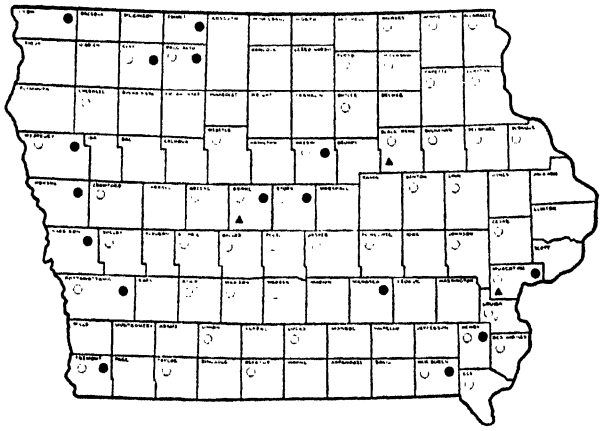
32. Distribution of *Euphorbia* (Poinsettia)

- ● E. dentata
- ○ E. heterophylla



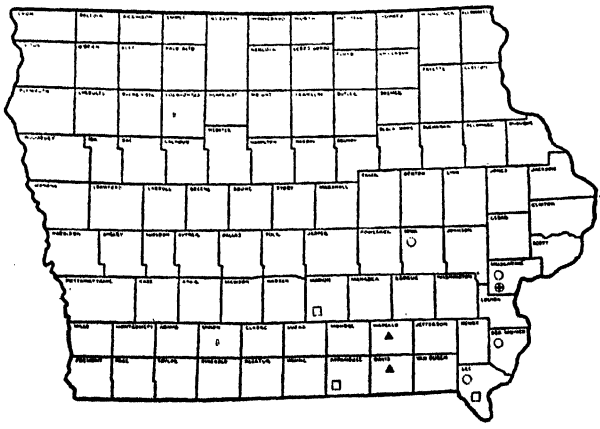
33. Distribution of *Euphorbia* (various)

- ● E. marginata
- ○ E. corollata
- ▲ E. hexagona



34. Distribution of *Croton*

- ○ C. glandulosus
- ▲ C. monanthogynous
- □ C. capitatus
- ⊕ C. texensis



Twenty-seven species and varieties occur in Iowa and appear in the following keys. All of these are represented in the Iowa State College or University of Iowa Herbaria. An additional one, *E. helioscopia* L., is included in the keys, because Iowa is in the range given by the manuals that cover the state.

The following species are not listed in Cratty's catalogue of Iowa plants (2) or the supplements (5, 6) by Hayden. An asterisk indicates specimens in the University of Iowa Herbarium.

Acalypha gracilens Gray

Rocky woody slopes along the Des Moines River near Cliffland, WAPELLO Co., Oct. 1, 1938, Ada Hayden 9154; Muscatine Island, MUSCATINE Co., Aug. 1896, F. Reppert*; Keokuk, LEE Co., July 5, 1895, B. Shimek.* The last cited specimen possibly represents a variety of gracilens.

Croton texensis (Klotzsch) Muell.

Along railroad track, Front Street, Muscatine, MUSCATINE Co., Sept. 1897, F. Reppert*.

Euphorbia platyphylla L.

EMMET Co., Aug. 11, 1894, R. I. Cratty*. This may have been a cultivated specimen. The manuals give the distribution of this species as northeastern United States, the Great Lakes to Manitoba.

The specimens in the Iowa State College Herbarium labelled *Euphorbia lucida* Waldst. & Kit. (2) prove to be *E. esula* L. So far as the writer knows, *E. lucida* does not occur in the state.

Euphorbia dentata Michx. was first reported for Iowa by Hayden (5). The following additional data add to the distributional picture. Loess soil along a dry roadside north of Hamburg, Fremont Co., Sept. 19, 1940, Ada Hayden 3937; along railroad track, Summit Bridge, Winthrop, Buchanan Co., Aug. 28, 1942, Margaret Murley 1650; dry gravelly soil, open ground, near Patterson, Madison Co., 1942, Kate Le Mar 94; Muscatine Co., Aug. 1896, W. D. Barnes 889*; Ft. Dodge, Iowa, Webster Co., Oct. 3, 1904, O. M. Oleson.

Euphorbia geyeri Engelm. was also first reported for the state by Hayden (5) from Allamakee Co. One other location is reported here: Muscatine Island near sand mound, Muscatine Co., 1879, 1890, 1891, 1892, 1894, F. Reppert*.

Several species are infrequent in Iowa. The following are particularly uncommon being represented from only one county: *A. ostryaefolia* Riddell, *Euphorbia missourica* Raf. var. *intermedia* (Engelm.) Wheeler, *E. platyphylla, E. peplus* L., *E. commutata* Engelm. and *Croton texensis.*

The following terms are defined as used in these keys:

Apex—the hilum end of the seed.

Areola—a little, usually angular, space on the surface.

Base—the chalazal end of the seed.

Caruncle—an excrescence covering or near the hilum being of a spongy, fleshy membranous or cartilaginous nature. Being fragile, it is often lost and hence cannot always be used as a distinguishing character.

Chalaza—the point in an ovule or seed where the integuments diverge from the nucellus.

Facets—one of the sides or faces of quadrangular seeds.

Papillose—bearing slight protuberances.

Raphe—the ridge of an ovule extending from the hilum to the chalaza. In this family it appears as a ventral seam extending the length of the seed, ending in a concentric configuration at the base.

Reticulate—in the form of a network.

Striate—marked with fine ridges.

Tuberculate—with rounded projections.

KEY TO GENERA OF *EUPHORBIACEAE*

A. Seed ovoid with a pronounced pointed apex, 1.8 mm. in average length; seed *coat with fine to deep longitudinal striations or rugose wrinkles;* capsule glabrous, strigose or hairy. *Acalypha*

AA. Seed ovoid, ovoid-oblong, quadrangular, lenticular, turtle-shaped or sub-globose; seed *coat with transverse wrinkles or ridges, or tuberculate, reticulate, papillose or smooth.*

B. Seed ovoid, oblong, quadrangular (exceptions in *Euphorbia obtusata, E. dictyosperma,* and *E. platyphylla* which have seeds lenticular but are 2 mm. or under in length), .75 to 2.7 mm. in length (except in *E. marginata* which have seeds 4.3 mm. in length but possess tuberculate coats); seed *coat wrinkled, ridged, tuberculate, reticulate, papillose or smooth;* capsule glabrous, strigose, granular or wrinkled. *Euphorbia*

BB. Seed lenticular, turtle-shaped or sub-globose, 2.8 to 4.5 mm. in length; seed *coat almost smooth, appearing polished:* capsule with stellate pubescence. *Croton*

The genus *Acalypha*

The three-valved capsule may be glabrous, strigose or spiny. In shape, the seeds have an ovoid contour with a pronounced pointed apex. The average length of the seed is 1.8 mm. The seed coat is finely to deeply striated longitudinally or rugosely wrinkled longitudinally. Their color varies from a bright red to brownish-red to grey often with silver and grey mottlings. The caruncle is spongy and lip-like.

KEY TO SPECIES OF *ACALYPHA*

A. Seed 1.5 to 2 mm. in length; seed *coat striated longitudinally.*

B. Seed ovoid, 1.6 to 1.8 mm. in length; seed *coat with fine distinct striations,* fine cross bars (with a magnification of 30x) giving a netted appearance, coat may be heavily mottled, merely a few flecks of black or no mottling; capsule smooth to strigose.

Acalypha rhomboidea[5] (Fig. 1)
or *Acalypha virginica*[6]

BB. Seed globose-ovoid, 1.5 to 2 mm. in length; seed *coat with wavy and deeper striations,* cross bars not as prominent, frequently mottled; capsule strigose
Acalypha gracilens (Fig. 2)

[5] *Acalypha virginica* L. of recent manuals. (16) No distinguishing characters could be found to separate the seeds of *A. rhomboidea* Raf. from *A. virginica* L.

[6] *Acalypha digyneia* Raf. of recent manuals (16)

AA. Seed 1.8 to 2 mm. in length; seed *coat roughened by deep wrinkled furrows;* capsule with scattered spines and stiff hairs.
Acalypha ostryaefolia (Fig. 3)

The genus *Euphorbia*

The seed may be sharply angled, ovoid, ovoid-oblong or lenticular. In length the species in this genus range from .75 to 4 mm. An ashy to brown scurvy epidermis, in various stages of sloughing, covers the seed. This outer layer of mucilage or epidermis termed by Pammel (12), the outer coat, gives to many of the species a white or ashy color with the red, brown or otherwise colored testa showing through. If the seed coat is wrinkled or ridged, it is transversely so in contrast to the longitudinal striations of *Acalypha*. One exception is *Euphorbia supina* which possesses fine longitudinal striations on the testa. These strations are often obscured by the outer seed coat with its transverse wrinkles. The three-lobed capsule may be glabrous, strigose, granular or wrinkled.

KEY TO SPECIES OF *EUPHORBIA*

A. Seed quadrangular, sub-quadrangular to almost ovoid, lenticular or oblong, *.75 to 1.8 mm. in length* (exception *E. platyphylla* 1.9 to 2 mm.)

B. Seed *sub-quadrangular with rounded edges (sometimes almost ovoid), or sharply quadrangular;* caruncle minute or absent.

C. Seed *sub-quadrangular with rounded edges or sometimes almost ovoid,* .75 to 1.8 mm. in length.

D. Seed sub-quadrangular, .75 to 1 mm. in length; seed *coat wrinkled.*

E. Seed .75 mm. in length; seed *coat with faint transverse wrinkles, unbroken across the facets, fine longitudinal striations sometimes visible, tannish-white;* capsule strigose.
Euphorbia supina (Fig. 4)

EE. Seed 1 mm. in length; seed *coat with broken transverse wrinkles,* lead-grey; capsule glabrous.
Euphorbia maculata (Fig. 5)

DD. Seed almost ovoid, 1 to 1.8 mm. in length; seed *coat smooth to microreticulate.*

F. Seed slightly angled, 1.3 to 1.8 mm. in length seed *coat smooth,* outer coat white with the orange-red testa often showing through giving a mottled appearance.

G. Seed 1.3 mm. in length.
Euphorbia geyeri (Fig. 6)

GG. Seed 1.8 mm. in length.
Euphorbia missourica var. *intermedia*[1] (Fig. 7)

FF. Seed obtusely angled, 1 mm. in length; seed *coat microreticulate.*
Euphorbia serpens (Fig. 9)

CC. Seed *sharply quadrangular,* 1 to 1.3 mm. in length; seed coat white with the orange to tan testa often showing through.

H. Seed round pointed at the apex; seed *coat strongly ridged transversely.*
Euphorbia glyptosperma (Fig. 10)

HH. Seed long pointed at the apex; seed *coat smooth, faintly wrinkled or pitted.*
Euphorbia serpyllifolia var. *genuina* (Fig. 11)

[1] *Euphorbia petaloidea* Engelm. of some manuals.

BB. Seed *lenticular or oblong;* caruncle present.

I. Seed lenticular; seed coat smooth to reticulate.

J. Seed 1.5 to 1.75 mm. in length; seed *coat reticulate,* reticulations form a straight line on the dorsal side similar to a seam.

K. Seed *coat faintly reticulate forming areolae but with smooth areas,* brown; capsule possessing structures varying from scale-like to elongated warts.

Euphorbia obtusata (Fig. 13)

KK. Seed *coat with distinct reticulations, areolae open and regular,* purplish-brown; capsule with elongated warts.

Euphorbia dictyosperma[8] (Fig. 14)

JJ. Seed 2 mm. in length; seed *coat smooth,* reddish-brown, lustrous; capsule with depressed warts.

Euphorbia platyphylla (Fig. 12)

II. Seed *oblong;* seed coat pitted.

K. Seed 1.5 mm. in length; seed *coat with pits in rows, those on ventral facets nearly always elongated,* color ash-grey; caruncle conical, white, large for size of seed; capsule lobes crested.

Euphorbia peplus (Fig. 17)

KK. Seed 1.8 mm. in length; seed *coat with pits of uniform size* and more numerous, blue-grey; caruncle thin and flattened; capsule lobes not crested.

Euphorbia commutata (Fig. 18)

AA. Seed oblong or ovoid, *2 to 2.7 mm. in length* (except *E. marginata* which has seeds 4.3 mm. in length).

L. Seed oblong; seed 2 to 2.3 mm. long; *seed coat smooth or minutely pitted.*

M. Seed *coat smooth to minutely pitted;* seed possessing a caruncle.

N. Seed *coat smooth, frequently mottled,* ashy, lead-grey, or orange; capsule smooth to coarsely granular.

Euphorbia esula (Fig. 20)

NN. Seed *coat minutely pitted, never mottled;* lead-grey to brownish-grey; capsule finely granular.

Euphorbia cyparissias (Fig. 21)

MM. Seed *coat scurfy with slightly depressed areas,* ash-grey to brown; seed lacking a caruncle, but possessing a cavity in the hilum region.

Euphorbia corollata (Fig. 16)

LL. Seed oblong or ovoid; seed *coat furrowed, tuberculate or reticulate.*

P. Seed *oblong,* 2.5 mm. in length; seed coat papillose, yellow, with a reddish or darkened area at the base and apex.

Euphorbia hexagona (Fig. 19)

PP. Seed *ovoid.*

Q. Seed 2.5 to 4.3 mm. in length; seed *coat tuberculate.*

R. Seed *2.3 to 2.7 mm. in length;* tubercles coarse, rough, high, abundant, brown to black; capsule glabrous.

S. Seed sharply angular, 2.3 to 2.5 mm. in length; *tubercles irregularly placed and on the average larger; caruncle conspicuous and somewhat* 3-sided.

Euphorbia dentata (Fig. 23)

[8] *Euphorbia dictyosperma* Fisch. & Mey. (7)
Euphorbia arkansana var. *missouriensis* Norton of some manuals.

SS. Seed only slightly angular, 2.5 to 2.7 mm. in length; *some of the tubercles in a transverse row or rows on the dorsal side of seed,* caruncle minute or absent.
Euphorbia heterophylla (Fig. 24)

RR. Seed *4.3 mm. in length,* possessing a wide protruding ridge at the apex; tubercles not as high or abundant, often running into ridges, white to light tan; capsule pubescent.
Euphorbia marginata (Fig. 22)

QQ. Seed 2.2 mm. in length; seed *coat with high sharp reticulations forming 5-sided cells,* lead-grey; caruncle broad and thin.
Euphorbia helioscopia (Fig. 15)

The genus *Croton*

The seeds are borne in globose to oblong-ovoid capsules 4–6 mm. in length. The capsules may be two or three-celled and one or three-seeded, respectively. The stellate pubescence, distinctive of the genus, is found on all parts of the plant, including the capsule. The seeds may be subglobose, lenticular, or convex on dorsal side and with two beveled faces on the ventral side. In the early stages of maturity, the seed coat is striated to granular, and light in color. When fully mature, the seeds are lustrous, appearing smooth and polished, and darker in color. The position of the caruncle is well above the hilum leaving the latter structure in full view.

KEY TO THE SPECIES OF *CROTON*

A. Seed sub-globose or lenticular, *4 to 4.5 mm. in length;* caruncle frequently extending out in peg fashion away from the seed.

B. Seed *sub-globose;* seed coat almost smooth, lustrous, appearing polished, brown, frequently variegated.
Croton capitatus (mature) (Fig. 25a)

BB. Seed *nearly lenticular, ventral face not as convex as dorsal face,* seed coat finely striated to granular, lusterless, orange-tan, not variegated.
Croton capitatus (immature) (Fig. 25b)

AA. Seed lenticular, *2.8 to 3.8 mm. in length;* caruncle compact with a circular contour.

C. Seed *2.8 to 3 mm. in length;* seed coat minutely striated to almost smooth, polished, dark brown, frequently mottled with red.
Croton monanthogynous (Fig. 26)

CC. Seed *3.5 to 3.8 mm. in length;*

D. Seed convex on dorsal side, ventral surface with two faces somewhat curved, 3.8 mm. in length; seed *coat non lustrous, frequently with a bloom, reddish-brown, not mottled.*
Croton texensis (Fig. 27)

DD. Seed convex on dorsal side, ventral surface with two beveled or nearly beveled faces, 3.5 mm. in length; seed *coat highly lustrous, light brown, mottled with black.*
Croton glandulosus var. *septentrionalis* (Fig. 28)

The writer wishes to express her thanks to Dr. R. H. Porter and Dr. G. J. Goodman for sympathetic assistance in carrying out the problem, and also her appreciation to Dr. Duane Isely for testing the keys.

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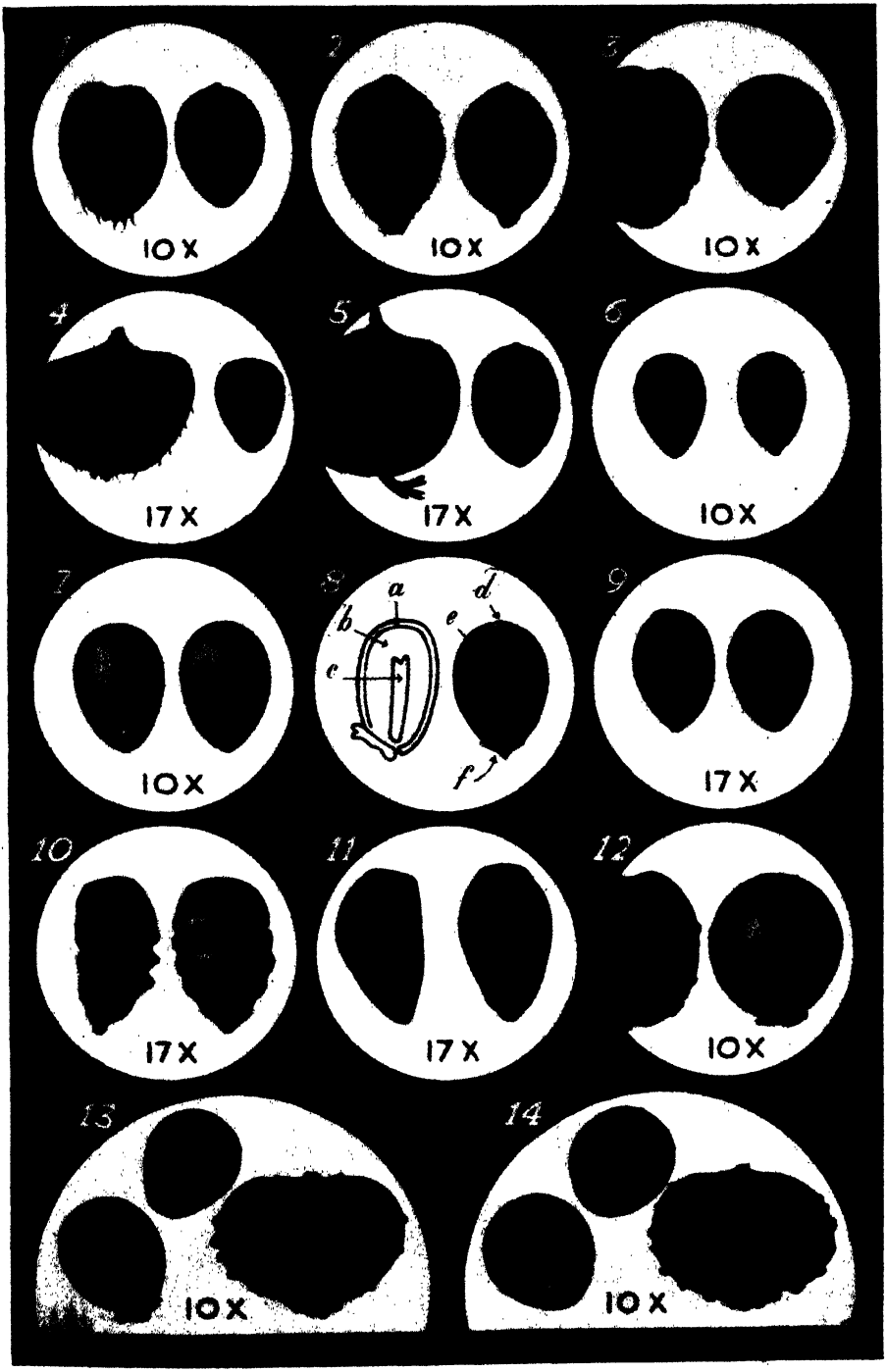
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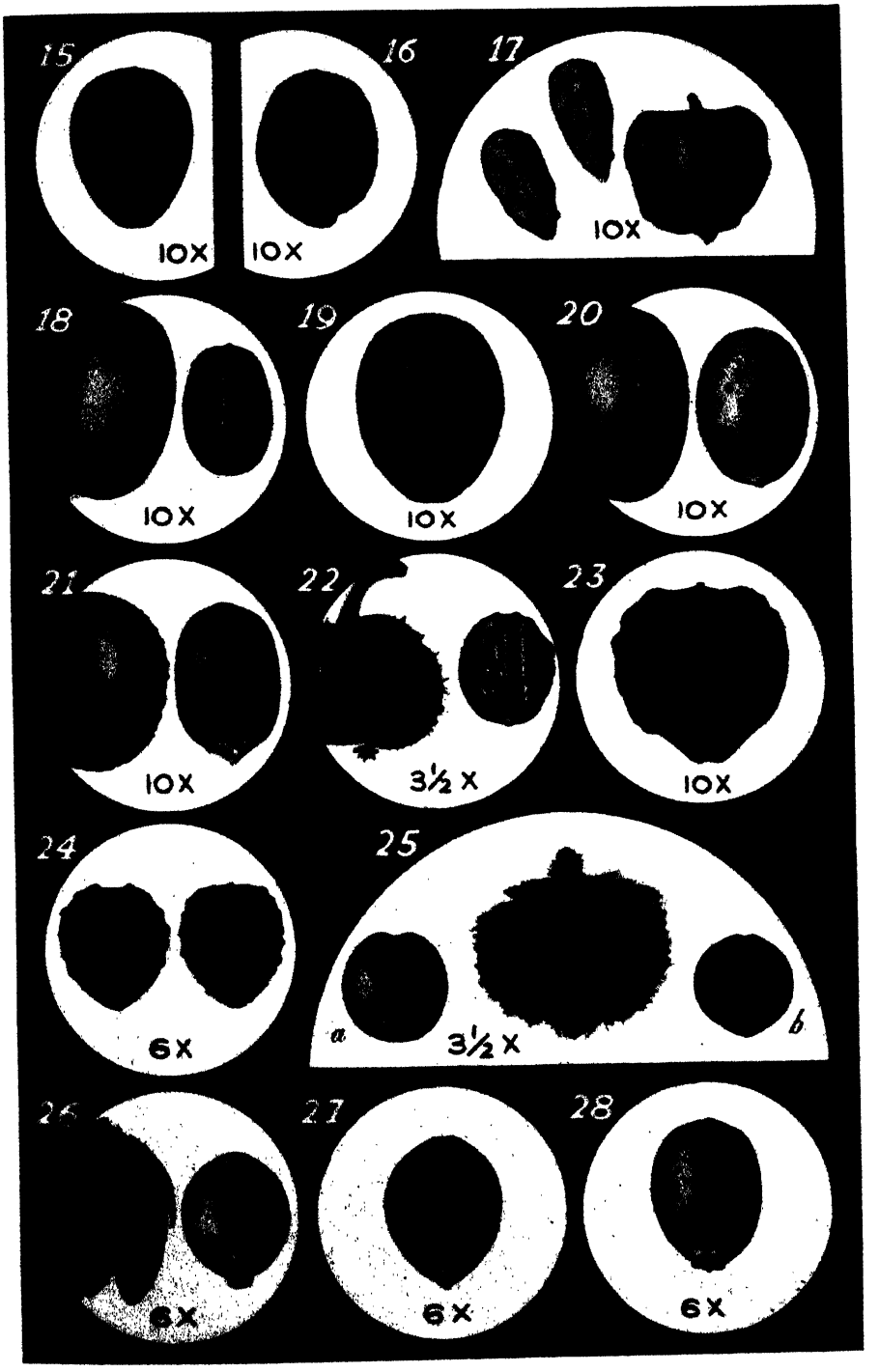
EXPLANATION OF FIGURES

Drawings by George Morris

1. *Acalypha rhomboidea* Raf. or *Acalypha virginica* L. Carpel and seed.
2. *Acalypha gracilens* A. Gray. Carpel and seed.
3. *Acalypha ostryaefolia* Riddell. Carpel and seed.
4. *Euphorbia supina* Raf. Capsule and seed.
5. *Euphorbia maculata* L. Capsule and seed.
6. *Euphorbia geyeri* Engelm. Dorsal and ventral views of seed.
7. *Euphorbia missourica* Raf. var. *intermedia* (Engelm.) Wheeler. Ventral and dorsal views of seed.
8. Representative seed.
 Longitudinal section at right angles to the flat face.
 a. seed coat, b. endosperm, c. embryo
 Ventral view of seed.
 d. chalaza, e. raphe, f. caruncle
9. *Euphorbia serpens* H B K. Dorsal and ventral views of seed.
10. *Euphorbia glyptosperma* Engelm. Lateral and ventral views of seed.
11. *Euphorbia serpyllifolia* (Pers.) var. *genuina* Boiss. Lateral and ventral views of seed.
12. *Euphorbia platyphylla* L. Portion of capsule, seed.
13. *Euphorbia obtusata* Pursh. Ventral and dorsal views of seed, capsule.
14. *Euphorbia dictyosperma* Fisch. & Mey. Ventral and dorsal views of seed, capsule.


1
10X
2
10X
3
10X
4
17X
5
17X
6
10X
7
10X
8
a
b
c
d
e
f
9
17X
10
17X
11
17X
12
10X
13
10X
14
10X

15. *Euphorbia helioscopia* L. Seed.
16. *Euphorbia corollata* L. Lateral view of seed.
17. *Euphorbia peplus* L. Ventral and dorsal views of seed, capsule.
18. *Euphorbia commutata* Engelm. Portion of capsule, seed.
19. *Euphorbia hexagona* Nutt. Seed.
20. *Euphorbia esula* L. Portion of capsule, seed.
21. *Euphorbia cyparissias* L. Portion of capsule, seed.
22. *Euphorbia marginata* Pursh. Capsule and seed.
23. *Euphorbia dentata* Michx. Seed.
24. *Euphorbia heterophylla* L. Ventral and dorsal views of seed.
25. *Croton capitatus* Michx. a. Mature seed, capsule, b. immature seed.
26. *Croton monanthogynous* Michx. Capsule and seed.
27. *Croton texensis* (Klotzsch) Muell. Arg. Seed.
28. *Croton glandulosus* L. var. *septentrionalis* Muell. Arg. Seed.


15
10X
16
10X
17
10X
18
10X
19
10X
20
10X
21
10X
22
3½ X
23
10X
24
6X
25
a
3½ X
b
26
6X
27
6X
28
6X

FURTHER TOXICITY STUDIES WITH THE DOG TICK DERMACENTOR VARIABILIS (SAY)[1]

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Inasmuch as labor and transportation difficulties precluded the possibility of field trials on tick control in the summer of 1943, as suggested by Tauber *et al* (3), a few further laboratory tests were performed, using dog ticks, *Dermacentor variabilis* (Say), collected locally at Ames. All ticks were obtained by "flagging," a method which results in little if any injury to the collected animals; all were adults, and none had fed in the adult stage. Control ticks, as well as experimental specimens after treatment, were confined singly in small vials plugged with cotton and kept in a constant temperature cabinet at 23° ± 1° C., with a relative humidity of about 80 per cent. Ticks were examined at 2, 24, 48, and 72 hours. Although ticks frequently remain without movement for some time, bringing the vial close to a warm light bulb elicited responsive movement from living individuals.

When insecticides in solution or suspension were used, ticks were picked up individually by grasping an appendage with forceps, dipped momentarily in the liquid, placed on paper toweling for a few seconds, then confined in individual vials; or 10 ticks at a time were placed in a small screen wire cage, or were folded inside a thin strip of cotton, and held under the surface of the liquid for a known length of time, then removed and placed individually in vials.

EXPERIMENTAL

Rotenone. The washing or immersing of dogs in derris dip for control of ticks is recommended by Bishopp and Smith (1). They suggest a preparation made by adding 1 ounce of mild soap and 2 ounces of fine derris or cube powder containing at least 3 per cent of rotenone to 1 gallon of warm water; such a dip would contain 0.045 per cent of rotenone. To test the effect on *D. variabilis* (Say) of rotenone dips, derris dust containing 5 per cent rotenone was added in various amounts to soap solution made by adding 0.75 grams of mild soap (Palmolive beads) to 100 cc. of distilled water. Derris powder did not remain in suspension long; the liquid was stirred before each immersion of ticks.

[1] Journal Paper No. J-1264 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 570.

[2] Now with the U. S. Public Health Service.

Results of thus subjecting ticks to the action of rotenone are shown in Table 1. For comparison, mortality of controls is presented in Table 2.

Table 2 shows that the mortality among those ticks subjected to plain water or soap solution dips exhibited mortality percentages not essentially different from entirely untreated specimens. The higher death rate figures of Table 1 must then be attributed to the action of the rotenone.

Ticks subjected to rotenone tended to be inactive, even when response to heat indicated that life was unquestionably still present. Careful checking of each specimen was necessary; cursory examination would have given erroneously high percentages of mortality.

TABLE 1

MORTALITY OF UNFED ADULT TICKS EXPOSED TO ROTENONE IN SOAP SOLUTION

No. of Ticks	5 Per Cent Derris Dust per 100 cc. Water	Equivalent Per Cent of Rotenone	Per Cent Soap	Duration of Exposure	Per Cent Dead at 2 hrs.	24 hrs.	48 hrs.	72 hrs.
100....	0.9 gms.	0.045%	0.75%	2 min. (in cotton)	2	13	34	51
50....	1.5 gms.	0.075%	"	Momentary direct contact	0	12	46	74
100....	1.5 gms.	0.075%	"	2 min. (in cotton)	0	8	27	54
50....	1.5 gms.	0.075%	"	5 min. (in cotton)	2	8	24	56
50....	3.0 gms.	0.15 %	"	Momentary direct contact	0	42	76	98
100....	6.0 gms.	0.3 %	"	2 min. (in cotton)	0	10	47	59

It will be noted that direct dipping of ticks resulted in higher mortality than when only a thin layer of cotton surrounded the specimen. Neither increasing the concentration of rotenone from 0.045 per cent to 0.3 per cent nor increasing the length of exposure from 2 to 5 minutes was effective in significantly increasing mortality. The reasons for this difference in results of treatment seem obscure. The folding in cotton procedure was used in an attempt to approach, even remotely, the position of a tick under a thick mat of hair, as found on some breeds of dogs.

Just how long a time different dog owners or handlers would give to washing or dipping a dog in the recommended rotenone treatment (1) is a matter of guesswork. However, the present experiments indicate that Bishopp and Smith's suggestions regarding rubbing the dip into the fur and allowing the fluid to dry on the animal are well worth following for the reason that the 0.045 per cent rotenone-equivalent preparation seems to be down near the level where its effectiveness toward dog ticks

may be negligible if carelessly or improperly applied. It should be pointed out also that the suspended particles settle out rapidly, and care should be taken to keep the rotenone-soap preparation well agitated while in use.

Nicotine sulfate. According to Bishopp and Smith (1), tick-infested vegetation is rendered relatively free of ticks for about 3 days by spraying the plants with a solution composed of 1 part nicotine sulfate (40 per cent nicotine), 1 part soap, and 288 parts water. Strong (2) also reports nicotine sulfate sprays to be effective in controlling the American dog tick, with a 90 per cent reduction apparent 24 hours after applying the spray.

In the series of tests here described, various amounts of 40 per cent nicotine sulfate were added either to distilled water or to mild soap solutions. American dog ticks confined in small wire cages were then

TABLE 2

MORTALITY OF CONTROL UNFED ADULT TICKS, UNTREATED, OR EXPOSED TO WATER OR SOAP SOLUTION

No. of Ticks	Treatment	2 hrs.	24 hrs.	48 hrs.	72 hrs.
280	Untreated	0	1	2	4
50	Dipped momentarily in water	0	0	0	6
100	Dipped in water for 2 min. in screen cage	0	1	1	1
50	Dipped for 2 min. in 0.75% soap solution, in cotton	0	0	0	4
50	Dipped for 2 min. in 0.75% soap solution, in screen cage	0	0	0	2

immersed in the solution, as described above. Also, 320-mesh dusting sulfur was impregnated with nicotine sulfate by dissolving the latter in absolute alcohol and mixing the resulting solution thoroughly into the sulfur, which was then dried and screened. This powder was dusted over ticks confined in a tall bell jar dusting tower (3). Results from these two types of tests are given in Table 3. Reference to Table 2 will show the comparison of mortality of these experimental ticks with controls.

Nicotine sulfate appears to act more rapidly than does rotenone and to be more effective as a dip than as a dust. The 0.138 per cent spray is equivalent to the mixture suggested by Bishopp and Smith (1). Apparently it would be effective as a tickicide if the pests can be given a thorough wetting during the application of the spray.

Miscellaneous compounds. Commercial grade sodium silicofluoride (du Pont), 30 per cent micronized crystox dust (Shell Oil), and a dust composed of 19 parts exhausted pyrethrum flowers and 1 part Dowicide

TABLE 3

MORTALITY OF UNFED ADULT TICKS EXPOSED TO NICOTINE SULFATE

No. of Ticks	Chemical	Equivalent Per Cent of Nicotine	Treatment	Per Cent Dead at			
				2 hrs.	24 hrs.	48 hrs.	72 hrs.
100	0.167% nicotine sulfate (40%), 1.0% soap, in water	0.069%	Dipped for 2 min. in screen cage	79	83	86	88
100	0.33% nicotine sulfate (40%), 1.0% soap, in water	0.138%	"	87	98	98	98
100	5.0% nicotine sulfate (40%), in water	2.0 %	"	100			
50	Sulfur, impregnated with 5% nicotine sulfate (40%)	2.0 %	2 gms. dusted in tower	44	52	58	60

(Penick) were found relatively ineffective in killing ticks when used in a dusting tower as described above.

SUMMARY AND CONCLUSIONS

1. Unfed adults of the American dog tick *D. variabilis* (Say) dipped directly into suspensions containing less than 1 per cent of rotenone showed a high mortality. The lethal effects did not appear promptly; none of the ticks had died 2 hours after exposure to 0.075 per cent or 0.15 per cent rotenone dips. Such effects were well evidenced, however, 3 days after treatment, with 74 and 98 per cent mortalities. Untreated controls exhibited only 4 per cent mortality in the same period.

2. The protection afforded by the fibers of even a very thin layer of cotton appeared to lessen the effectiveness of the rotenone against ticks, even with longer exposures than that afforded by momentary direct dipping. Whereas the latter treatment, with a 0.075 per cent suspension, resulted in 74 per cent mortality after 3 days, the former produced, with the same strength of solution, only 54 per cent mortality for 2 minutes of exposure, and 56 per cent for 5 minutes 3 days after treatment. It is suggested that the hair of dogs might also offer protection to ticks harbored during rotenone dip treatments, unless the suspension is rubbed into the coat.

3. Nicotine in equally low concentrations in water appeared to be highly effective in killing American dog ticks and to act more rapidly than did rotenone. Seventy-nine per cent of the ticks held for 2 minutes in a 0.069 per cent nicotine solution were dead at the end of 2 hours. All those similarly treated with a 2 per cent nicotine solution died within 2 hours.

4. Ticks were more susceptible to equivalent amounts of nicotine in dips than in impregnated sulfur dusts.

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COMPARATIVE STUDIES OF SOME PERITRICHOUS PHYTOPATHOGENIC BACTERIA[1]

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Classification of the bacterial plant pathogens, more than three decades a topic of lively debate, has undergone drastic changes in recent years. A vast literature covering intensive studies on various groups of bacteria of importance to public health, disease, preservation of food, and certain manufacturing processes, has provided a mass of data which showed the older systems of bacterial classification inadequate. Subsequently the classification schemes of Lehmann and Neumann (1896) and Migula (1900), which were used by plant pathologists, were discarded by American bacteriologists, and a concerted effort to devise a more inclusive system was undertaken. Important contributions to this end were made by Buchanan (1916-1918), Winslow *et al.* (1917, 1920), and Bergey (1923), whose *Manual of Determinative Bacteriology* marked the culmination of these efforts. The disposition of the bacterial plant pathogens in Bergey's classification was discussed by Burkholder (1930, 1939). The European taxonomic ideas concerning the plant pathogens were ably presented by Stapp (1935) and Dowson (1939).

In the process of revising the classification of the bacterial plant pathogens, unfortunately, considerable confusion arose; many commonly used generic names carried more than one connotation, depending upon which system was followed. Despite the efforts of Erwin F. Smith (1905) and Buchanan (1916-1918) to place bacterial nomenclature on a more sound basis, progress in clearing up the confusion has been, for the most part, disappointing. The practical inadequacies of all systems of bacterial classification have been brought into sharp focus by the critical reviews of Breed (1928), Rahn *et al.* (1929), Kluyver and van Niel (1936), Dowson (1939), Elliott (1943), and many others.

The present study was initiated in 1937 to investigate the relationships of the peritrichous plant pathogens to other bacteria with the thought that perhaps a more reliable basis for their classification and nomenclature might be devised. The study was conducted with 78 isolates of as many species of peritrichous bacterial plant pathogens as could be collected

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from various sources. These isolates, together with a few nonmotile pathogens, were studied comparatively with cultures of allegedly closely related nonpathogenic species.

ORIGIN AND PATHOGENICITY OF CULTURES

The cultures used in the study were obtained from type culture collections and individual workers in many parts of the world. Whenever possible, type cultures of each species were obtained. Before the studies were begun, the cultures were purified by plating several times and their pathogenicity or identity determined. Unless the identity of a culture could be established beyond doubt it was discarded. This purification of cultures was important, because in some instances cultures exhibiting unusual reactions were found to be mixtures of two or more organisms, one the true pathogen and the others nonpathogenic contaminants. In other instances cultures were simply incorrectly identified. For example, a number of nonpathogenic strains of coliform bacteria were received under the labels of *Erwinia carotovora* and *E. phytophthora*.

Table 1 contains a brief summary of the available information concerning the cultures included in the study; their species designation when received; their origin and the symptomatic responses when inoculated into various host materials. The isolates were taken from a wide range of host plants in widely scattered localities. Represented in the study were type cultures of *Erwinia salicis* (No. 80), *E. lathyri* (No. 29), *E. carotovora* (No. 20), *E. phytophthora* (No. 23), *E. aroideae* (No. 24), and *E. melonis* (No. 25). The culture received as the type of *E. solanisapra* (EP) was identified as a strain of *Aerobacter aerogenes*. Some of the type cultures were isolated by the workers who named the species in question, and throughout the study these cultures retained most of the distinguishing characteristics described some 30 or 40 years ago.

All the cultures of *Erwinia amylovora*, except culture No. C-77s, produced characteristic symptoms on succulent apple twigs and green pear fruits. The nonpathogenic strain exhibited characteristic laboratory reactions which were accepted as proof of its identity. Isolates of *E. tracheiphila* always produced characteristic wilt symptoms in curcurbit plants within 10 days after inoculation. The three strains of *E. salicis* were not tested on Salix species, but their identity was satisfactorily established by noting their characteristic production of a lemon yellow pigment in potato media.

Forty-two cultures of "soft-rot" bacteria, bearing six species names, together with several unnamed strains were studied. Organisms isolated locally were assigned tentative species designations. With the exception of cultures W48, W51, W53, CA1, CA2, P4, EP, and 496, all of the "soft-rot" cultures exhibited various degrees of pathogenicity when introduced into raw carrot slices, potato tubers, and potato stems. The symptomatic response always was pectolytic in nature.

Most of the nine cultures of *E. dissolvens* studied exhibited a rather doubtful pathogenicity on corn plants but were satisfactorily identified by their cultural reactions.

The two cultures of *E. lathyri* (Nos. 28 and 29) were nonpathogenic on all host plants tested and are probably not the same species described by Manns (1915). The culture of *E. ananas* (No. 101) was acquired only a short time before the study was terminated and was not tested on the host of its origin, the pineapple.

The identities of the coliform cultures (*Escherichia coli, E. freundii, Aerobacter aerogenes,* and *A. cloacae*), *Serratia marescens,* and *Proteus vulgaris* were established by their characteristic reactions in appropriate cultural media.

CROSS INOCULATION STUDIES

The most satisfactory method for conducting the crossinoculations was by hypodermically introducing a bacterial cell suspension obtained by washing the bacteria from a 2- or 3-day-old agar culture in 20 cc. of distilled water into actively growing plants in the greenhouse. The inoculations were repeated from one to six times.

The fruits and vegetables were inoculated hypodermically and incubated in moist chambers at room temperatures (26 ± 2°C.). Raw carrot slices were placed in petri dishes containing two layers of water-soaked filter paper and inoculated by placing a loopful of bacterial suspension in a drop of water on the top of each carrot slice. No organism was considered a "soft-rot" organism unless it caused a visible softening of the carrot slices within 48 hours. Such organisms will be referred to as the "pectolytic bacteria" throughout this paper. Any softening appearing after 48 hours was considered to be due to fermentation processes rather than exoenzymatic pectolysis. Such softening seldom was observed.

The results of the crossinoculations, summarized in Table 1, show a tendency toward obtaining successful inoculations most often from the hosts related to those from which the cultures were isolated. The pectolytic bacteria exhibited a much wider host range than did *Erwinia amylovora* and *E. tracheiphila.*

The various isolates of pectolytic bacteria showed rather wide and constant differences in their ability to invade the stems and leaves of potato, tobacco, and cucumber plants. The relative virulence and symptomatic responses exhibited by the various strains usually were found to be fairly reliable distinguishing characteristics which proved to be valuable aids in differentiating species. For example, in almost every trial, culture No. 20 was noticeably less virulent than culture No. 24. In general, the gas-forming strains were not as virulent as the nongas-formers.

With potato plants there appeared to be a definite tendency for some isolates to produce blackleg symptoms as a characteristic reaction, whereas other isolates never were observed to produce the blackening typical of the disease. These differences were not correlated with virulence. Some

of the nonblackleg-forming cultures exhibited extraordinary virulence, frequently killing the plant with 3 or 4 days. Cultures producing blackleg symptoms often failed to kill the plant by the end of 3 weeks after inoculation. In the light of these observations and the reports found in the literature, it is strongly suspected that *E. solanisapra,* studied by the workers in England and by Stapp in Germany, may have been a similar nonblackleg-forming pectolytic organism of high virulence for potato stems.

Several cultures received as strains of *E. carotovora* (W48, W51, W52, W53, CA1, CA2), *E. phytophthora* (P4, 496), and *E. solanisapra* (EP) were found to be nonpathogenic on all the host plants tested and, except for culture, No. 496, were identified as members of the coliform bacteria. These so-called nonpathogenic pathogens were retained for study in order to determine simple methods for distinguishing the true from the contaminating cultures.

The cultures of corn stalk-rot bacteria (*E. dissolvens*) were found to be so slightly pathogenic on corn plants that for a time it was thought that they should probably be considered nonpathogenic contaminants. Culture W71, however, produced restricted watersoaked lesions in the midribs of the leaves of four out of seven corn plants inoculated. Later, culture No. 100 was found to have a noticeable degree of virulence when it was first received, but in later inoculation trials its pathogenicity was apparently lost. Since the purification procedure revealed the presence of only one type of colony, the loss of pathogenicity could not be attributed to a contaminant overrunning the organism in question. Stanley (1938) and Elrod (1941), who conducted studies with these same isolates, believed that the corn stalk-rot bacteria might belong to the pectolytic bacteria, which were considered to be strains of coliform organisms. That this is not the case is evident from the results of the crossinoculations recorded in Table 1 and also from Rosen's (1926) original description of *Bacterium dissolvens* and Jones' (1901) description of *Bacillus carotovorus.*

In none of the crossinoculation studies did *Aerobacter aerogenes, A. cloacae, Escherichia coli, E. freundii, Serratia marcescens,* or *Proteus vulgaris* produce visible evidence of ability to invade living plant tissues. These observations are considered evidence that the contention that species of Erwinia are strains of coliform bacteria is untenable.

The crossinoculation studies indicated first that *Erwinia amylovora* and *E. tracheiphila* are highly host specific; secondly, that the pectolytic organisms are able to attack a relatively wide range of host plants by virtue of their propectinase activity; thirdly, that the virulence of the pectolytic bacteria on potato plants is not correlated with their ability to produce blackleg symptoms; fourthly, that cultures designated as "nonpathogenic soft-rot or blackleg bacteria" usually are readily identified as coliform organisms; fifthly, that the corn stalk-rot organisms are not pectolytic bacteria; and sixthly, that the yellow organisms received as *E. lathyri* (Nos. 28, 29) are in all probability not the same as the organism described by Manns (1915).

TABLE 1

ORIGIN OF CULTURES, DESIGNATION WHEN RECEIVED, AND SYMPTOMATIC RESPONSES WITH VARIOUS SELECTED HOST MATERIALS

Culture No.	Origin of Cultures						Symptomatic Responses With Selected Hosts†							
	Designation When Received	Received From*	Source	Host	Place	Date Isolated	Green Pear	Apple Twig	Cucumber Plant	Carrot Slices	Tobacco Plants	Corn Plants	Potato Tuber	Potato Plants
31	*Erwinia amylovora*	E. M. Hildebrand	Fire blight				3Z	2	0	0	0	0	0	0
32	" "	" "	" "				2	2	0	0	0	0	0	0
33	" "	" "	" "				1	2	0	0	0	0	0	0
34	" "	" "	" "	Green crab			1	1	0	0	0	0	0	0
35	" "	G. C. Kent	Fruit rot	Jonathan	Iowa	1939	3Z	3	0	0	0	0	0	0
36	*Erwinia amylovora*	A. J. Riker	Canker exudate	Dudley	Wisconsin	1932	3Z	2	0	0	0	0	0	0
37	" "	" "	Blossom blight	"	"	1932	3Z	3	0	0	0	0	0	0
82	" "	Local isolate	Twig blight	*Pyrus serotina communis*	Iowa	1938	3Z	3	0	0	0	0	0	0
83	" "	" "	" "	"	"	1938	3Z	3	0	0	0	0	0	0
84	" "	" "	" "	*Crataegus monogyna*	"	1938	3Z	2	0	0		0	0	0
85	*Erwinia amylovora*	G. C. Kent	Twig blight	Wealthy	Iowa	1940	3Z	3	0	0			0	
86	" "	" "	" "	Yellow transparent	"	1940	3Z	3	0	0			0	
C-SC	" "	P. A. Ark	Fire blight	*Pyrus communis*	S. Carolina	1935	2	1	0	0		0	0	
C-77a	" "	" "	" "	*malus*	"	1935	0	0	0	0	0	0	0	0
C-50	" "	" "	" "	*Pyracantha angustifolia*	California	1929	1	1	0	0		0	0	0
C-501	*Erwinia amylovora*	P. A. Ark	Fire blight	*Crataegus oxyacantha*	New York	1931	1	1	0	0		0	0	0
C-507	" "	" "	" "	*crus-galli*	"	1931	0	1	0	0			0	
C-55	" "	" "	" "	*Pyracantha angustifolia*	California	1933	3Z	3	0	0		0	0	0
C-55.2	" "	Reisolate of C-55	Fruit inoculation	*Pyrus communis*	Iowa	1940	3Z	3	0	0		0	0	
C-64	" "	P. A. Ark	Fire blight	*Photinia arbutifolia*	California	193"	3Z	2	0	0		0	0	0
94	*Erwinia tracheiphila*	S. Doolittle	Wilt	Cucumber	Maryland	1937	0	0	3W	0	0	0	0	0
95	" "	" "	" "	"	"	193"	0	0	3W	0	0	0	0	0
96	" "	" "	" "	"	"	1938	0	0	3W	0	0	0	0	0
79	*Erwinia salicis*	W. J. Dowson	Watermark	*Salix alba*	England		0	0	0	0	0	0	0	0
80	" "	" "	" "	" *coerulea*	"		0	0	0	0	0	0	0	0
81	" "	" "	" "	" *fragilis*	"		0	0	0	0	0	0	0	0
28	*Erwinia lathyri*	W. J. Dowson	Diseased insects	Aphis	England		0	0	0	0	0	0	0	0
29	" "	Nat. Type Cult. Col.	" "	"	"		0	0	0	0	0	0	0	0
101	" *ananas*	R. P. Elrod	Brown fruitlet rot	Pineapple	Malaya					0			0	0
17	*Erwinia carotovora*	J. G. Leach	Blackleg	Potato			0	0	0	2	1	0	2	1
18	" "	G. L. McNew (B60)	" "	"	Minnesota		0	0	0	3	1	0	2	1
M60	" "	" "	" "	"	"		0	0	0	3	1	0	2	1
19	" "	" " (B12)	Soft-rot	Carrot	New Jersey	1935	0	0	0	3	1	0	3	1
M12	" "	" " "	"	"	"	1935	0	0	0	3	1	0	3	1
20	" "	A.T.C.C. (No. 495)	"	"	Vermont	1898	0	0	0	2	0	0	2	1
21	*Erwinia carotovora*	J. G. Leach	Blackleg	Potato	Minnesota		0	0	0	2	2		3	1
22	" *aroideae*	Reisolate of K2	Carrot inoculation	Carrot	Iowa	1940	0	0		3	3		3	3B
23	" *phytophthora*	A.T.C.C. (No. 496)	Blackleg	Potato	Germany	Before 1909	0	0	0	2	1	0	2	2B
24	" *aroideae*	A.T.C.C. (No. 494)	Corm decay	Calla	Maryland	1912	0	0	3	3	3	0	3	3B
25	" *melanis*	A.T.C.C. (No. 920)	"			Before 1926							3	2B
26	" "	R. P. Elrod	Soft-rot	Turnip	New Zealand					2			3	3B
27	*Erwinia aroideae*	R. P. Elrod	Soft-rot	Carrot	Rhode Island	1938				3			3	1B
75	" "	Local isolate	Corm decay	Cyclamen	Iowa	193"	0	0	2	3	3	0	3	3B
M10	" *carotovora*	G. L. McNew (B10)	Soft-rot	Carrot	New Jersey	1935	0	0	3	3	3	0	3	3B
M81	" *aroideae*	G. L. " (B81)	"	*Brassica petsai*	Japan	1931	0	0	1	2	2	0	3	
K1	" "	E. M. Johnson	Blackleg	Tobacco	Kentucky	193"	0	0		3	3	0	3	3B
K2	" "	" "	" "	"	"	1938	0	0		3	3	0	3	1
C8	*Erwinia phytophthora*	P. A. Ark	Bacterial blight	Larkspur	California	1936				2	2	0	1	1
C10	" *carotovora*	" " "	Soft-rot	Carrot	"	193"				3	2	0	2	2B
C11	" *aroideae*	" " "	Fruit rot	Squash	"	193"				3	3	0	2	1B
C12	" *carotovora*	" " "	"	"	"	"				2	2	0	2	1
W1	" *aroideae*	E. A. Martin	Blackleg	Potato	Minnesota					3	1	0	3	1B
W2	" "	" "	"	"	"					2	1	0	1	1
W3	*Erwinia atroseptica*	E. A. Martin	Blackleg	Potato						2		0	2	1
W13	" "	" " "	Soft-rot	Canna	West Virginia	1932				3		0	3	1
W20	" "	" " "	"	Potato	" "	1932				3		0	2	1
W38	" "	" " "	Soft-rot	Turnip	" "	1932				3		0	2	2B
W42	" "	" " "	"	Pe Tsai	Japan					2	3	0	3	2B
W120	" "	" " "	"	Potato	West Virginia	1933				2		0	2	1B
106	*Erwinia aroideae*	R. P. Elrod	Fruit rot	Pumpkin	California	1938				3			3	3B
107	" "	Local isolate	Blackleg	Potato	Iowa	1940				3			3	3B
108	" "	" "	Tuber rot	"	"	1940				3			3	3B
109	" "	" "	"	"	"	1940				3			3	3B
110	" "	R. Rustigian	(See No. 24)							2			1	
111	" "	" "	Soft-rot	Carrot	Rhode Island	1938				3			3	
112	" "	" "	"	Potato	"	1938				3			3	
W48	*Erwinia carotovora*	E. A. Martin			Illinois		0		0	0	0	0	0	0
W51	" "	" " "			"		0			0	0	0	0	0
W52	" "	" " "			"		0			0	0	0	0	0
W53	" "	" " "			"		0			0	0	0	0	0
CA1	" "	R. P. Elrod			"					0			0	0
CA2	" "	" "			"					0			0	0
EP	*solanisapra*	" "	Tuber rot	Potato	Brazil	Before 1928				0			0	0
P4	" *phytophthora*	" "	"	"	Ohio	1939				0			0	0
496	" "	R. Rustigian	(See No. 23)	"	"					0			0	
W4‡	*Erwinia dissolvens*	E. A. Martin	Stalk rot	*Zea mays*			0	0	0	0	0	0	0	0
W5	Stalk-rot organism	" " "	" "	" "	West Virginia	1931	0	0	0	0	0	0	0	0
W6‡	"	" " "	" "	" "	" "	1931	0	0	0	0	0	0	0	0
W7	"	" " "	" "	" "	" "	1931	0	0	0	0		1	0	0
W68	*Erwinia dissolvens*	" " "	" "	" "			0	0	0	0		1	0	0
W70	Stalk-rot organism	" " "	" "	" "	West Virginia	1931	0	0	0	0		1	0	0
W71	"	" " "	" "	" "	" "	1932	0	0	0	0		2	0	0
W119	"	" " "	" "	" "	" "	1932	0	0	0	0		1	0	0
100	"	(Retrieved)	"	"	Indiana	1940			0	0		3	0	0
201	*Aerobacter aerogenes*	C. H. Werkman	Soil				0	0	0	0	0	0	0	0
202	" *cloacae*	" "	Sewage						0	0		0	0	0
203	*Escherichia coli*	C. H. Werkman	Urinary tract	Man			0	0	0	0	0	0	0	0
204	" *freundii*	" "	Canal water		Holland				0	0			0	0
205	*Serratia marcescens*	M. Levine							0	0	0	0	0	0
206	" *Proteus vulgaris*	" "							0	0			0	0

* The generosity of the many individuals who contributed cultures used in this study is gratefully acknowledged.

† Scale for estimate of infection:
0 = no visible symptoms
1 = slight infection
2 = moderate infection
3 = severe infection
– = not determined
Z = ooze
W = wilt
B = blackleg

‡ Two cultures of Isolates No. W4 and W6 were used.

CULTURAL STUDIES

MORPHOLOGY AND STAINING REACTIONS

Motility: Motility of the cultures was determined by the hanging drop and semi-solid agar methods (Tittsler and Sandholzer, 1936). Two to 3-day-old broth cultures were used for hanging drop examination and for the inoculation of the semisolid agar tubes. The semisolid agar stab cultures were examined 24, 48, and 72 hours after inoculation for evidence of motility. All of the isolates of *E. amylovora, E. salicis, E. tracheiphila, E. lathyri,* and *E. ananas* were found to be actively motile by both methods of examination. Most of the pectolytic isolates were found to be motile, but a few of the organisms failed to exhibit motility on some occasions. The matter of motility, or lack of it, of these isolates was not studied further. Of the corn stalk-rot organisms only cultures No. W71 and 100 were found to be nonmotile. Discrepancies of this sort were noted by Rosen (1922, 1926) for these bacteria. *Serratia marcescens, Aerobacter cloacae, Escherichia coli,* and *E. freundii* were motile, whereas *Aerobacter aerogenes* and *Proteus vulgaris* were not.

Flagellation: Flagella stains were made on only a few isolates. The Casares-Gil (Plimmer and Paine, 1921) and Leifson (1930) methods were used. Smears of cultures Nos. 20, 28, 36, 75, 79 and 81 showed unmistakable peritrichous flagella two to three times the length of the somatic cell, varying in number from four to eight. It was not thought worthwhile to pursue this aspect further, since the flagellation of this group of bacteria is well established, and also because flagellation is of secondary importance in making the comparisons with the other bacteria studied.

Form and size: Observations on form, size, etc., of the isolates were made on smears stained with the gram and acid-fast stains. The cells of all the isolates had rounded ends and were of uniform shape. All cells were rod-shaped, although some strains had cells so short as to appear almost coccoid. Rather marked differences in length were observed among the various strains of a given species. Detailed measurements were not made on all the isolates, since Ark's (1937) work with *Erwinia amylovora* and Stapp's (1928) painstaking studies on the blackleg and soft-rot organisms seemed to be adequately confirmed by the observations made. Moreover, it was noted that the size of the cells of a single culture varied widely from one time to the next; bearing out the observations reported by others that cell size of a strain of bacteria is markedly influenced by the medium and conditions under which it was cultured, and is, therefore, not a particularly satisfactory character to use in classification.

The isolates of *E. tracheiphila* were conspicuous in that the cells were considerably longer than those of the other bacteria studied, being two to five times as long as they were wide.

Gram stain: The gram staining was done by Buchanan's anilin gentian violet method (Levine, 1933) and Reed's rapid gram stain (Racicot, *et al.*, 1938). All of the organisms studied were Gram-negative by both methods.

Smears of *Bacillus subtilis* stained at the same time were always Gram-positive.

Acid-fast stain: The acid-fast staining was carried out using the Ziehl-Neelson method described in the S.A.B. *Manual of Methods* (1936). None of the organisms was found to be acid-fast. *Mycobacterium phlei* (Moeller) Bergey preparations stained at the same time were invariably acid-fast.

Spore formation: No evidence of endospore formation was obtained in any of the cultures of bacteria included in the study.

PHYSIOLOGY AND FERMENTATION REACTIONS

Detailed descriptions were not worked out from the growth characteristics of all the cultures. Only those strikingly distinguishing characters noted from time to time in routine culture media were recorded.

Nutrient agar slants: The pectolytic bacteria produced a rather more copious growth than did *Erwinia amylovora, E. salicis,* and *E. tracheiphila.* But none of these bacteria grew as luxuriantly as the coliform bacteria and the corn stalk-rot organisms. Except for *E. lathyri, E. ananas,* and *Serratia marcescens,* all the bacteria produced greyish-white to cream-colored growths on nutrient agar slants. *Erwinia lathyri* and *E. ananas* were yellow; *Serratia marcescens* was red. None of the cultures was observed to produce diffusible pigments in the agar. All the bacteria, except *Erwinia tracheiphila,* which always produced a slimy growth, produced growths of butyrous consistency. In all the cultures studied, the surface of the growth was smooth, and dull to shining by reflected light. The smooth-rough phases, although appearing from time to time, were not studied in detail.

Potato dextrose agar slants: All of the cultures grew much more luxuriantly on potato dextrose agar slants than on nutrient agar. *E. amylovora* grew rapidly but tended to die after 6 to 7 days on this enriched medium. A characteristic lemon yellow pigment produced by *E. salicis* on potato dextrose agar was a very convenient diagnostic character. *E. tracheiphila* grew more luxuriantly and was cultured much more easily on this medium than on nutrient agar.

The corn stalk-rot and coliform bacteria produced luxuriant growths and usually produced gas in amounts sufficient to cause great cracks to form in the butts of the slants. The pectolytic bacteria likewise grew luxuriantly but did not produce gas in amounts sufficient to crack the agar.

Bouillon: In bouillon and other liquid culture media, while wide differences in turbidity, sedimentation and pellicle formation were observed among the various cultures, these differences had no value in distinguishing the various species in question. The cultural characters in bouillon were found to be typical of the reactions described for the species by other workers.

Eosin-methylene blue and Endo agar plate cultures: To determine whether the bacteria studied exhibited growth characters typical of the coliform organisms, platings were made on Levine's eosin-methylene blue

agar and Endo agar plates. The plates were examined 48 hours and 1 week after inoculation.

E. amylovora produced very small, white to pale pink colonies 1 to 4 mm. in diameter, which did not in the least resemble those of the coliform bacteria. *E. salicis* grew poorly on these media, producing pin-point colonies that resembled those of *E. amylovora*. In three trials *E. tracheiphila* failed to grow on these media.

The pectolytic bacteria produced colonies that were usually smaller than those of the coliform bacteria but produced the metallic sheen characteristic of the Escherichia species, never shiny as were the Aerotobacter colonies. In general appearance the colonies of the pectolytic bacteria did not closely resemble those of either section of the coliform bacteria, however. The corn stalk-rot organism produced colony types that were identical with those of *Aerobacter aerogenes* and *A. cloacae*.

The "nonpathogenic soft-rot and blackleg" bacteria produced colony types typical of *Aerobacter* and the "intermediate" sections of the coliform bacteria. *Erwinia lathyri* and *E. ananas* were not grown on E.M.B. and Endo agar plate cultures.

Reduction of Nitrates: Reduction of nitrates has been in use as a bacteriological test for many years. With due consideration of the many difficulties incident to the effective use of the nitrate-reduction tests pointed out by Conn (1936), the behavior of the plant pathogens with respect to nitrates was considered of fundamental importance in this study. Buchanan (1916-1918) used nitrogen metabolism to a considerable extent as the basis for establishing his major taxonomic categories. The value of nitrogen metabolism as a fundamental criterion in the classification of bacteria was emphasized by Knight (1938). Kent (1942) reported that *E. amylovora*, *E. salicis*, and *E. tracheiphila*, three nonnitrate-reducing organisms, were unable to sustain visible growth in media containing inorganic nitrogen compounds when included with an appropriate carbon source.

In several trials *E. amylovora*, *E. tracheiphila*, *E. salicis*, and *E. ananas* did not reduce nitrates. All the isolates of pectolytic bacteria, the coliform bacteria, the corn stalk-rot bacteria, *Serratia marcescens*, and *Proteus vulgaris* reduced nitrates to nitrites without formation of visible gas.

Production of Hydrogen Sulphide: In the present study the ability of the phytopathogenic bacteria to produce hydrogen sulphide was determined by growing the organisms in two media, namely, the proteose peptone-ferric citrate agar medium of Levine *et al.* (1932) and a liquid medium of the same composition, except that the ferric citrate and agar were omitted. Lead acetate-impregnated paper strips were suspended over the liquid cultures as hydrogen sulphide indicators. The cultures were examined 1, 2, 7, and 14 days following incubation. The following arbitrary measure of hydrogen sulphide production was found to be useful:

0 = no blackening in either medium.
1 = slight blackening of lead acetate paper after 2 days; none in agar.

2 = marked blackening of lead acetate paper the first day; none in agar.

3 = marked blackening of both lead acetate paper and agar the first day.

The summarized results of the hydrogen sulphide studies are presented in Table 2. *Erwinia amylovora, E. tracheiphila,* and *E. salicis* failed to produce hydrogen sulphide in quantities sufficient to blacken lead acetate papers during the period of incubation. *E. salicis* tended to turn the lead acetate papers yellow after several days, but the reaction was so slight that it was considered to be negative. All the isolates of *E. lathyri,* the pectolytic bacteria, the corn stalk-rot bacteria, and the coliform bacteria (*Escherichia freundii* excepted) produced hydrogen sulphide sufficient to blacken the indicator strip suspended over the liquid medium but not sufficient to blacken the agar medium. *E. freundii* produced blackening in the ferric citrate agar.

These results indicate that *Erwinia amylovora, E. tracheiphila,* and *E. salicis* apparently lack the mechanism present in the other bacteria studied for releasing hydrogen sulphide from the proteoses. With due consideration given to the composition of the medium and to the technique used in detecting the presence of hydrogen sulphide, this reaction appears to have considerable taxonomic merit. The pectolytic and corn stalk-rot bacteria produced hydrogen sulphide in amounts similar to the coliform bacteria.

Litmus Milk Reactions: Litmus milk cultures were incubated 30 days and observations made 1, 2, 5, 10, 20, and 30 days after inoculation. Changes in reaction, curd formation, and reduction of the indicator are summarized in Table 2.

E. amylovora was the only plant pathogen that showed any proteolytic activity in milk culture coincident with the production of an alkaline reaction. *E. tracheiphila* and *E. salicis* brought about no visible changes in litmus milk cultures, a characteristic which was a very useful means for identifying these organisms. The pectolytic and corn stalk-rot bacteria produced reactions nearly identical with those of the coliform bacteria. Acid formation occurred within 24 to 48 hours, followed by reduction of the indicator and formation of a firm curd with or without extrusion of whey.

The litmus milk reactions suggest a rather close relationship between the coliform and pectolytic bacteria. Culture No. 28 appears to be unrelated to any of the other bacteria in its behavior toward skimmilk. That *E. amylovora* is distinct from *Proteus vulgaris* is indicated by the rapid peptonization and strong alkali production of the latter in contrast with the relatively weak reactions of the former.

EXOENZYMATIC ACTIVITY

Gelatin liquefaction, starch hydrolysis, and peptonization of milk are essentially estimates of exoenzymatic activity. Of these only the starch-hydrolyzing enzyme system, amylase, has been extensively studied. In the present study no attempt was made to identify the end products of the enzyme action or to determine the linkages involved. The hydrolytic activities of the bacterial plant pathogens in living plant tissues, starch,

gelatin, and milk, were referred to as protopectinase, amylase, and proteinase acting on gelatin and milk.

Protopectinase: The secretion of protopectinase by the pectolytic bacteria was first studied by Potter (1901) and later by Jones (1909). Kertesz (1936), in his discussion of the literature relating to the cell wall-dissolving enzyme system, pointed out that while it is not definitely proven that protopectinase, pectinase, and pectase are distinct enzyme systems, the evidence in the literature seems to favor the view that the cell wall-dissolving enzyme system, protopectinase, is a distinct component of the pectinase complex; its activity is manifested by maceration of living plant tissue and conversion of insoluble protopectins to soluble pectins.

The test for protopectinase activity was conducted according to the method described for the crossinoculation studies. Pectolysis was determined after 48 hours incubation. To check whether the softening of plant tissues by the "soft-rot" bacteria was due to exoenzymatic activity, alcohol precipitates were prepared from 500 ml. bouillon cultures of *Erwinia carotovora* (No. 20), *E. phytophthora* (No. 23), *E. aroideae* (Nos. M10 and K1), *E. amylovora* (Nos. 36 and 37), *Escherichia coli*, and "pectin-fermenting" coliform culture received under the name *Aerobacter pectinovorum*. The dried precipitate was ground with zinc dust in a mortar and dissolved in 10 ml. of water. After centrifuging the redissolved enzyme preparation, the supernatant enzyme solution was tested for protopectinase activity by suspending in it a very thin slice of carrot. Maceration of the carrot was taken as evidence of protopectinase activity. Active preparations were obtained only from pathogenic cultures of *Erwinia carotovora*, *E. phytophthora*, and *E. aroideae*. The cultures of *E. amylovora*, *Escherichia coli*, and *Aerobacter pectinovorum* yielded no trace of protopectinase activity. This experiment strongly supported the idea that plant tissue inoculation is a reliable test for protopectinase, the directness and simplicity of which requires no defense.

In view of the importance of protopectinase in the parasitism of many pathogenic plant organisms, the ability of bacteria, in particular, to secrete this enzyme should be considered highly significant in characterizing and classifying bacteria. For this reason the results of the raw carrot slice test for determining protopectinase activity have been included with the other three enzyme tests in Table 2.

The summarized results of Table 2 show that only the pectolytic bacteria were able to secrete protopectinase in amounts sufficient to soften raw carrot slices in 48 hours. Cultures received as "nonpathogenic" strains of *Erwinia carotovora* (W48, W51, W52, CA1, CA2), *E. phytophthora* (P4, 496), and *E. solanisapra* (EP), on the other hand, failed to show the slightest evidence of protopectinase activity. These "nonpathogenic" soft-rot organisms were also different from the pathogenic cultures in that they failed to liquefy gelatin, a property which was characteristic of all the pectolytic bacteria studied.

Amylase: In the present study amylase activity was tested by means of starch agar plate cultures, supplemented by a soluble starch-peptone broth. A weak iodine solution was used to test for hydrolysis.

With the exception of a few cultures of corn stalk-rot bacteria, the bacterial plant pathogens listed in Table 2 produced detectable amylase activity. These observations, together with the fact that most of the bacterial plant pathogens do not ferment maltose, give rise to some interesting speculations concerning the presence or absence of an alphaglucosidase system in the enzyme complex of the various organisms in question.

Proteinase activity in gelatin: In this study no attempt was made to determine whether the proteinases responsible for liquefaction of gelatin were distinct from those peptonizing litmus milk. Tubes containing nutrient gelatin were inoculated by a needle stab from actively growing bouillon cultures and incubated at 21 ± 1°C. for 30 days. Observations were made after 1, 2, 5, 15, and 30 days incubation. Visible liquefaction was recorded as "proteinase activity."

E. lathyri, Serratia marcescens, and all the isolates of pectolytic bacteria showed visible liquefaction of gelatin within 24 to 48 hours. At the end of 30 days many of the cultures had completely liquefied the gelatin in the tubes; others were from one-half to three-fourths liquefied. The liquefaction of gelatin was but one of several cultural and biochemical characters that these three groups of bacteria exhibited in common. Cultures of *Erwinia amylovora* liquefied gelatin slowly, but their gelatin-liquefying activity was much more vigorous than that of the corn stalk-rot bacteria, *Aerobacter cloacae* or *Proteus vulgaris.* The "nonpathogenic" cultures of *Erwinia carotovora* (W48, W51, W52, W53, CA1, CA2), *E. phytophthora* (P4), and *E. solanisapra* (EP), when tested for proteinase activity in gelatin stab cultures, failed to liquefy gelatin, further evidence that these organisms are not pectolytic but coliform bacteria. No proteinase activity was produced by *E. tracheiphila, E. salicis, Aerobacter aerogenes, Escherichia coli,* and *E. freundii* in gelatin cultures by the end of the 30-day incubation period.

Proteinase Activity in Milk: The proteinase activity of the various cultures in milk was determined by noting the amount of digestion produced in litmus milk cultures after 2, 5, 15, and 30 days incubation at 29 ± 1°C. The only organisms that showed visible digestion were the *Erwinia amylovora* isolates and *Proteus vulgaris.* In no instance, however, was the proteinase activity of *Erwinia amylovora* as strong as that of *Proteus vulgaris* when grown in milk cultures.

The results in Table 2 suggest that the proteolytic enzymes of the bacterial plant pathogens acting on gelatin and milk proteins might be two specific enzyme systems. The pectolytic bacteria produced rapid proteolysis of gelatin but no visible digestion of litmus milk. The cultures of *Erwinia amylovora* and *Proteus vulgaris,* on the other hand, showed proteolytic activity in both media. Although the specificity reported by Diehl (1919) seems to be supported by the results summarized in Table 2, the proteolytic activity of the pectolytic bacteria in litmus milk cultures may have been inhibited by the rapid formation of acids in the milk. While it is not certain that the differences in proteolytic activity observed are due to different proteinase systems, it was convenient, for the purpose of

classification, to consider the proteolytic activities observed in gelatin and milk separately.

THE "IMViC" REACTIONS

The mnemonic "IMViC", proposed by Parr (1936), designates the four most commonly used tests for characterizing members of the coliform bacteria, namely, (I) indole production, (M) methyl red reaction, (V) V-P reaction, and (C) Koser's citrate utilization test. With the exception of the indole test, none of these tests has been extensively applied to studies of the bacterial plant pathogns. Consquntly, it is almost impossibl to determine from the literature the relationships between the phytopathogenic and coliform bacteria.

Indole Production: In the present study the indole test was made according to the recommendations outlined in the S.A.B. *Manual of Methods* (1936). Each bacterial isolate was grown in duplicate tubes of a 1 per cent solution of sugar-free Bacto Tryptone for 5 days at 29 ± 1°C. Using the Gore cotton plug technique with the Ehrlich-Bohme reagents, the cultures were tested for indole after 2 and 5 days incubation.

The results are briefly summarized in Table 2. None of the plant pathogens studied produced indole under the conditions described. *Escherichia coli, E. freundii,* and *Proteus vulgaris* produced strongly positive reactions, whereas *Aerobacter aerogenes, A. cloacae* and *Serratia marcescens* were consistently negative.

In a series of studies of indole-production among 80 strains of *Bacterium stewartii, Pseudomonas campestre, Ps. phaseoli, Ps. phaseoli* var. *fuscans, Ps. pisi, Ps. lespedezae,* and several other species of vascular bacterial plant parasites, not a single indole-producing strain was found. Apparently indole production among bacterial plant pathogens is of rare occurrence.

Methyl red reaction: The methyl red test has been used only to a limited extent in studying the bacterial plant pathogens. Stanley (1938), Dowson (1939), and Elrod (1942), in their comparative studies, made use of the test but reported no striking correlations. In the present study the methyl red reaction was determined essentially according to the original recommendations of Clark and Lubs (1915). Vaughn *et al.* (1939) emphasized the importance of adhering strictly to these original recommendations. The medium used contained 0.5 per cent K_2HPO_4. Tubes containing Clark and Lubs' medium were inoculated from 2 to 3-day-old agar cultures and incubated at 29 ± 1°C. for 5 days.

The results of the methyl red tests are summarized in Table 2. With the exception of some of the pectolytic bacteria, the reaction was frequently variable and indecisive. The marked tendency for the phytopathogenic bacteria to produce intermediate methyl red reactions (yellow-orange to orange-red) indicates that the zone of limiting pH for the bacterial plant pathogens probably does not coincide with that of any of the three classes of coliform bacteria. The failure of *Erwinia amylovora, E. tracheiphila, E. salicis* and the pectolytic bacteria to respond to the

methyl red test in the precise manner characteristic of the Aerobacter and Escherichia species is taken as evidence that these plant pathogens are not closely related to either section of coliform bacteria.

The "nonpathogenic" cultures of *Erwinia carotovora* (W48, W51, W52, CA1, CA2), *E. phytophthora* (P4), and *E. solanisapra* (EP) usually gave methyl red reactions identical with those of coliform bacteria.

The Voges-Proskauer Reaction: The Voges-Proskauer reaction has not been extensively used in characterizing the bacterial plant pathogens. Ark (1937) and Dowson (1939) reported a V-P reaction for *E. amylovora.* Dowson (1939) reported *E. tracheiphila* and *E. carotovora* to be V-P negative and *E. salicis, E. aroideae* and *E. lathyri* to be V-P positive. Stanley (1938) obtained both positive and negative V-P reactions for the pectolytic bacteria studied by him, but the bacteria isolated from corn stalk-rot were all found to be V-P positive. Millard (1924) and Metcalfe (1940) found *Bacterium rhaponticum* to be V-P positive, and Chester (1938) reported a slightly positive V-P reaction for *Erwinia cytolytica.* Elrod (1942) also reported positive and negative V-P reactions for 19 soft-rot isolates studied by him. No V-P reaction has been reported for the other petritrichous plant pathogens.

The V-P reaction was determined by growing the organisms in Clark and Lubs' medium at 29 ± 1°C. Determinations were made after 48 hours incubation using 40 per cent NaOH and creatine as recommended by O'Meara (1931). After 1940, duplicate tests were made using 40 per cent KOH and alphanaphthol according to the method of Barritt (1936). Vaughn *et al.* (1939) observed that the number of so-called intermediate reactions could be materially reduced by carefully controlling conditions under which the tests were conducted. Careful attention, therefore, was given to the temperature and time of incubation in this study. During 1941 it was found desirable to make an additional determination after 5 days incubation. The cultures were always run in duplicate. After each test was made, cultures giving doubtful or unexpected reactions were plated out, and several isolated colonies, definitely known to be the organism, were retested.

The results of the V-P determinations are summarized in Table 2. It was apparent from the number of doubtful reactions obtained that the V-P reaction is not as clear-cut when used with the plant pathogens as it is with the coliform bacteria. This tendency indicates that the plant pathogens are not coliform bacteria. In general, it can be said that *E. amylovora, E. salicis,* and *E. tracheiphila* were V-P negative, although in some instances some of the cultures seemed to be slightly positive. Among the pectolytic bacteria, four isolates (Nos. 24, 27, M10, M81, W2) were considered to be definitely V-P positive. These include the type culture of *E. aroideae* (No. 24). The remainder of the pectolytic organisms were considered to be V-P negative, although there were some instances where the results were doubtful. The corn stalk-rot organisms all gave strikingly negative V-P reactions typical of the Aerobacter species.

The "nonpathogenic" soft-rot organisms (W48, W51, W52, W53, CA_1, CA_2, EP) gave reactions identical with the Aerobacter species of the

coliform bacteria. Culture No. P4 gave a strong positive V-P reaction typical of the Escherichia species.

From the standpoint of classification it is believed that the V-P reaction of the pectolytic bacteria is highly significant. By the balance sheet method of analysis, Birkenshaw *et al.* (1931) found considerable differences between two strains of *Erwinia aroideae* and *E. carotovora*. *E. aroideae* gave a very high yield of butylene glycol, equivalent to more than 40 per cent of the original sugar, and very little lactic acid, whereas *E. carotovora* gave no butylene glycol and a high yield of lactic acid, corresponding to about 40 per cent of the sugar consumed. Should this difference in metabolic activity be confirmed by more extensive studies with several strains of pectolytic bacteria, this would be adequate justification for recognizing the V-P reaction as a fundamental criterion for the classification of these organisms.

The Koser Citrate Utilization Test: Duplicate sets of Difco-dehydrated Koser citrate medium were inoculated from young (less than 1-week-old) agar slant cultures. After 2 days incubation at 29 ± 1°C. the tubes were examined for visible growth. At that time serial transfers to fresh citrate tubes were made; they were also examined after 2 days, when serial transfers were again made. An organism was considered "citrate-positive" only if visible growth occurred in the third serial transfer after 2 days incubation.

The results of the Koser citrate test are summarized in Table 2. With the exception of culture C8, all the pectolytic isolates produced abundant growth in 48 hours, in most cases a marked turbidity appearing within 24 hours. Of the nonpathogenic organisms, *Serratia marcescens*, *Aerobacter aerogenes*, *A. cloacae*, and *Escherichia freundii* produced strong positive reactions, whereas *E. coli* was negative. *Proteus vulgaris* gave negative results, but a suggestion of growth could be detected after 5 days. *Erwinia amylovora*, *E. tracheiphila*, and *E. salicis*, although sometimes producing a slight turbidity after 5 days, particularly with the first culture in the series, were not able to sustain visible growth in Koser's medium through three serial transfers. *E. ananas* and *E. lathyri* grew well in the medium.

For some time the tendency of some cultures to produce a faint turbidity, especially upon prolonged incubation, was a source of considerable concern. The indecisive results were eliminated by adopting the serial transfer technique. The results obtained in these experiments were consistent, however, with the results of Rucchoft *et al.* (1931) in their study of several coliform organisms. The cultures of *E. amylovora* were able to build up populations almost to the point of visible turbidity, but when serial transfers were made most of these cultures failed to maintain visible growth.

FERMENTATION REACTIONS

The interpretation of the literature pertaining to fermentation reactions is complicated by the tendency of various workers to use different techniques, not only in conducting the tests, but also in interpreting their

observations. This tendency has led many workers to question the value of the fermentation tube in bacterial identification and classification. Wedum (1936) pointed out, however, that despite the limitations of the acid-indicator fermentation tube technique, reliance can be placed on the results, provided no unwarranted assumptions are made in the interpretation of the observations. Furthermore, the use of synthetic basal media for fermentation studies (Ayers, Rupp, and Johnson, 1919; Burkholder, 1932) makes it difficult to effect equitable comparisons with organisms studied in peptone media.

Technique: In the present study the fermentation reactions were determined by growing the organisms in Durham tubes containing 0.5 per cent peptone, 0.1 per cent K_2HPO_4, and 0.1 to 0.5 per cent of the fermentable substance. Brom cresol purple was added as acid indicator at the rate of 1 ml. of 1.6 per cent alcoholic solution per liter of medium. Synthetic basal media were not feasible, because many of the isolates studied required peptone as a source of nitrogen and growth factors. The media were sterilized by autoclaving for 10 to 15 minutes at 15 pounds pressure. No advantage was gained by sterilizing the sugar solutions through a Berkfield filter and adding to the basal medium aseptically.

The fermentation tests were conducted in duplicate and repeated at least once. All fermentation tube cultures were incubated at 29 $\pm$ 1°C. for 2 weeks. The 2-week incubation period was found to be most satisfactory, since shorter incubation periods were not long enough to allow the slowest fermenters to produce visible changes in the acid indicator incident to their fermentative activities, and incubation periods longer than 2 weeks were found unnecessary. Any changes appearing after 2 weeks incubation were found to be due to the development of contaminants in the culture tubes.

The Fermentation Index: The customary method of determining the fermentation reactions by recording formation of acid on a simple qualitative basis was found to be inadequate, because the bacteria included in the study exhibited the widest variety of fermentative ability. Some isolates produced acid and gas quickly and in relatively large amounts, whereas others produced acid or gas slowly and in very small amounts. The quantitative differences in gas-production were found to be constant and highly significant from the standpoint of classification. To bring out these differences adequately, the following arbitrary scale for evaluating the fermentative ability of the organisms in Durham tube cultures was found to be valuable:

0—no visible acid or gas
?—acid formation doubtful, no visible gas
1—acid, no visible gas
X—acid, erratic gas production (none to 10 per cent in Durham tubes)
2—acid, slight gas production (less than 10 per cent in Durham tubes)
3—acid, abundant gas production (more than 10 per cent in Durham tubes)

Differences in fermentation rate were brought out by recording the

TABLE 2

GENERAL SUMMARY OF PATHOGENICITY, CULTURAL CHARACTERS, EXOENZYMIC ACTIVITIES, "IMViC," AND OTHER MISCELLANEOUS REACTIONS* OF SOME PERITRICHOUS AND NONMOTILE PHYTOPATHOGENIC BACTERIA AND SOME RELATED NONPATHOGENIC SPECIES

Culture No. (type)	Species Designation (type)	No. Isolates	Origin (host)	Pathogenicity	Cultural Characters: Agar	P.D.A.	E.M.B.	NO_3 to NO_2	H_2S Production	Litmus Milk†	Exoenzymic Activities: Protopectinase	Amylase	Proteinase: Gelatin	Milk	"IMViC" Reactions: Indole	M-R	V-P	Citrate
37	*Erwinia amylovora*	23	Rosaceous plants	0–3	white	white	pale pink	0	0	B	0	0	1	0–1	0	1	0	0
94	" *tracheiphila*	6	Cucurbit species	3	"	"	no growth	0	0	N	0	0	0	0	0	1	0	0
80	" *salicis*	3	Willow species		"	yellow	pale pink	0	0	N	0	0	0	0	0	1	0	0
20	*Pectobacterium cartovorum*	4	Fleshy vegetables	1–3	white	white	metallic	3	2	ACR	2–3	0	2–3	0	0	3	0	3
C12	" "	2	" "	1–3	"	"	"	3	2	ACR	2–3	0	2–3	0	0	3	0	3
23	" *phytophthorum*	3	Potato (usually)	1–3	"	"	"	3	2	ACR	2–3	0	2–3	0	0	3	0	3
17	" "	4	" "	1–3	"	"	"	3	2	ACR	2–3	0	2–3	0	0	3	0	3
24	" *aroideae*	5	Fleshy plants	1–3	"	"	"	3	2	ACR	2–3	0	2–3	0	0	1	3	3
25	" *melonis*	17	" "	1–3	"	"	"	3	2	ACR	2–3	0	2–3	0	0	1	0	3
W2	" "	1	" "	1–3	"	"	"	3	2	ACR	2–3	0	2–3	0	0	3	0	3
C8	" *dalphini*	1	*Delphinium* species	2	"	"	"	3	2	ACR	2–3	0	2–3	0	0	3	0	0
201	*Aerobacter aerogenes*	1	Soil	0	white	white	brown	3	2	ACR	0	2	0	0	0	0	3	3
W48	" "	7	Soft-rot of plants	0	"	"	"	3	2	ACR	0	. . .	0	0	0	0	3	3
202	" *cloacae*	1	Sewage	0	"	"	"	3	2	ACR	0	0	1	0	0	0	3	3
W4	" *dissolvens*	8	" "	0–2	"	"	"	3	2	ACR	0	0	0–1	0	0	0	3	3
100	" "	1	" "	0	"	"	"	3	2	ACR	0	2	0	0	0	0	3	3
203	*Escherichia coli*	1	Man	0	white	white	metallic	3	2	ACR	0	0	0	0	3	3	0	0
204	" *freundii*	1	Canal water	0	"	"	"	3	3	ACR	0	. . .	0	0	3	3	0	3
P4	" "	1	Blackleg of potato	0	"	"	"	3	. . .	ACR	0	. . .	0	0	0	3	0	3
205	*Serratia marcescens*	1	. . .	0	red	red	red	3	. . .	ACR	0	. . .	3	0	0	1	0	0
28	*"Bacillus lathyri"*	1	Sick aphis	0	chrome	yellow	. . .	3	2	ACR	0	0	3	0	0	0	0	0
29	" "	1	. . .	0	"	"	. . .	3	2	N-R	0	0	3	0	0	0	0	0
101	*Bacillus ananas*	1	Pineapple	. . .	"	"	. . .	0	. . .	. . .	0	. . .	0	0	0	3	0	0
206	*Proteus vulgaris*	1	. . .	0	white	white	. . .	3	. . .	B	0	. . .	0	3	3	3	0	1
496	*"Erwinia phytophthora"*	1	Blackleg of potato	0	"	"	. . .	. . .	. . .	. . .	0	. . .	0	0	0	. . .	. . .	. . .

* Intensity of reactions:
0 = no visible reaction
1 = slight or indecisive reaction
2 = moderate reaction
3 = strong reaction
. = not determined

† Litmus milk reactions:
A = acid reaction
B = basic reaction
N = neutral reaction
C = curd formation
R = reduction of indicator

TABLE 3

GENERAL SUMMARY OF FERMENTATION INDEXES* OF THE VARIOUS PERITRICHOUS AND NONMOTILE PHYTOPATHOGENIC BACTERIA AND SOME RELATED NONPATHOGENIC SPECIES AS DETERMINED FROM DURHAM TUBE CULTURES WITH 27 FERMENTABLE SUBSTRATES

Type Culture No.	Species Designation (type)	Pentoses: Arabinose	Xylose	Rhamnose	Aldohexoses: Glucose	Galactose	Mannose	Ketohexoses: Fructose	Sorbose	Oligosaccharides: Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Sucrose	Raffinose	Polysaccharides: Dextrin	Starch	Cellulose	Inulin	Glucosides: Esculin	Salicin	Glycerol	Erythritol	Dulcitol	Mannitol	Sorbitol	Inositol
37	*Erwinia amylovora*	0022	0002	0000	2111	0022	0000	0211	0000	0000	0000	0002	0000	0021	0211	0000	0020	0000	0000	0000	0022	0000	0022	0000	0000	0000	0000	0000
94	" *tracheiphila*	0000	0000	0000	0211	0011	0000	0011	0000	0000	0000	0000	0000	0000	0111	0000	0020	0000	0000	0000	0000	. . .	0000	0000	0000	1111	0000	0000
80	" *salicis*	0000	0000	0000	1xxx	0000	11xx	01xx	0000	0000	0000	0002	0000	0011	1xxx	0011	0000	0000	0000	0000	1111	0211	0022	0000	0000	0xxx	0000	0000
20	*Pectobacterium cartovorum*	1222	1x22	xx22	xxxx	1xx2	1122	xxxx	0000	0000	xxxx	?1xx	1xxx	xxxx	xxxx	xxxx	0000	0000	0000	0000	1xxx	1111	0001	0000	0000	xx22	0000	0000
C12	" "	. . .	. . .	. . .	1111	00?x	. . .	?211	. . .	0000	00xx	0002	00xx	1111	02?x	02xx	. . .	0000	0000	. . .	0001	0111	0001	. . .	. . .	. . .	. . .	. . .
23	" *lyphthorum*	1222	1222	1122	xxxx	0x22	1222	1xxx	0000	0000	xxxx	0x22	1x22	1111	xxxx	0xxx	0000	0000	0000	0000	xxxx	1xxx	0000	0000	0000	2222	0000	0000
17	" "	00xx	00xx	1x22	11xx	0002	02?x	02xx	0000	0000	0002	2222	00xx	2111	00xx	00xx	0000	0000	0000	0000	0001	0222	0000	0000	0000	02?x	0000	0000
24	" *aroideae*	1111	1111	1111	1111	2111	11??	?1??	. . .	0000	1111	1111	1111	1111	1111	1111	0000	0000	0000	0000	1111	1111	0222	0000	0000	1111	0000	0000
25	" *melonis*	1111	1111	1111	1111	1111	. . .	. . .	. . .	. . .	. . .	1111	1111	1111	1111	1111	0000	0000	0000	0000	1111	1111	0022	0000	0000	1111	0000	0000
W2	" "	. . .	. . .	. . .	0001	0002	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	0001	0000	. . .	. . .	. . .	. . .	. . .	. . .
C8	" *delphini*	. . .	. . .	. . .	xxxx	00xx	. . .	1111	. . .	0022	01xx	00xx	001x	0000	xxxx	0020	0000	0000	0000	. . .	1111	1111	0000	. . .	. . .	. . .	. . .	. . .
201	*Aerobacter aerogenes*	3333	3333	3333	3333	3333	3333	3333	. . .	3333	3333	. . .	3333	. . .	3333	3333	00x3	0x33	0000	. . .	3333	3333	3333	0000	0000	. . .	3333	1333
W48	" "	. . .	. . .	. . .	2333	. . .	. . .	3333	. . .	. . .	. . .	. . .	?2xx	. . .	3333	. . .	. . .	. . .	. . .	. . .	. . .	2332	. . .	. . .	. . .	. . .	. . .	. . .
202	" *cloacae*	. . .	. . .	. . .	3333	3333	. . .	2333	. . .	3333	3333	. . .	3333	. . .	0000	0000	. . .	0000	0000	. . .	0001	0021	0011	. . .	. . .	. . .	. . .	. . .
W4	" *dissolvens*	. . .	. . .	. . .	3333	3333	. . .	2333	. . .	3333	3333	. . .	00x3	. . .	2333	x333	. . .	0001	0000	. . .	3333	x22x	0011	. . .	. . .	. . .	. . .	. . .
W7	" "	. . .	. . .	. . .	3333	3333	. . .	2332	. . .	3333	3333	. . .	0012	. . .	3333	0000	. . .	0000	0000	. . .	3333	2220	0011	. . .	. . .	. . .	. . .	. . .
W70	" "	. . .	. . .	. . .	3333	3333	. . .	2333	. . .	3333	3333	. . .	0002	. . .	0000	0000	. . .	0001	0000	. . .	3333	2220	0011	. . .	. . .	. . .	. . .	. . .
100	" "	. . .	. . .	. . .	3333	3333	. . .	0233	. . .	3333	3333	. . .	0223	. . .	2333	3333	. . .	0?33	0000	. . .	3333	3322	3333	. . .	. . .	. . .	. . .	. . .
203	*Escherichia coli*	3333	3333	3333	3333	3333	3333	3333	0000	3333	0000	. . .	3333	1233	0000	0000	0000	0001	0000	0000	0122	01xx	0123	0000	0000	. . .	2333	0000
204	" *freundii*	. . .	. . .	. . .	3333	3333	. . .	3333	. . .	3333	3332	. . .	3333	. . .	2333	3333	. . .	0000	0000	. . .	3333	3332	0133	. . .	0000	. . .	3333	. . .
P4	" "	. . .	. . .	. . .	3333	. . .	. . .	. . .	. . .	3333	. . .	. . .	0033	. . .	3333	. . .	. . .	. . .	. . .	. . .	. . .	2322	. . .	. . .	. . .	. . .	. . .	. . .
205	*Serratia marcescens*	. . .	. . .	. . .	1111	0021	. . .	1111	. . .	1111	1000	. . .	0000	. . .	1111	0000	. . .	0011	0000	. . .	1111	1110	1111	. . .	. . .	1111	. . .	. . .
28	*"Bacillus lathyri"*	1111	1111	1111	1111	1111	1111	1111	0000	1111	0000	1111	0000	0111	1111	0000	0000	0000	0000	0000	1111	1111	0000	0000	0000	. . .	0000	0000
29	" "	1111	1111	1111	1111	1111	1111	1111	0000	1111	1111	1111	?111	0111	1111	0000	0022	0000	0000	0000	1111	1111	0089	0000	0000	. . .	0000	0000
101	" *ananas*"	. . .	. . .	. . .	1111	0111	. . .	1111	. . .	0110	. . .	. . .	. . .	. . .	1111	1111	. . .	. . .	0000	. . .	. . .	1111	. . .	. . .	. . .	. . .	. . .	. . .
206	*Proteus vulgaris*	. . .	. . .	. . .	2232	0000	. . .	0000	. . .	1222	0000	. . .	0000	. . .	1111	0000	. . .	0000	0000	. . .	1111	1111	0011	. . .	. . .	. . .	. . .	. . .
496	*"Erwinia phytophthora"*	. . .	. . .	. . .	0211	. . .	. . .	. . .	. . .	?111	. . .	. . .	0022	. . .	2222	. . .	. . .	. . .	. . .	. . .	. . .	3333	. . .	. . .	. . .	. . .	. . .	. . .

* Determination of fermentation indexes:
0 = no visible acid or gas
? = acid doubtful; no visible gas
1 = acid; no visible gas
x = acid; eventual gas production
2 = acid; slight gas production (less than 10%)
3 = acid; abundant gas production (more than 10%)
. . = not determined

First digit = reaction after 24 hours
Second digit = reaction after 48 hours
Third digit = reaction after 7 days
Fourth digit = reaction after 14 days

fermentation reactions after 24 hours, 48 hours, 1 week, and 2 weeks, consecutively, as a four-digit expression, which was called the "fermentation index." Thus *Escherichia coli,* which is notable for its vigorous lactose fermentation, can be said to have a lactose fermentation index of 3333. This means that within 24 hours *E. coli* produced acid and gas in excess of 10 per cent and that this reaction remained roughly within the prescribed bounds during the 2-week incubation period. *Serratia marcescens* had a galactose fermentation index of 00?1, that is, no acid was detectable until after 1 week, at which time the acid production was doubtful, but at the end of 2 weeks a definite acid reaction was discernible. *Erwinia carotovora* (No. 20) had a lactose index of 01XX, or, no acid was formed until 48 hours incubation, and after 1 week gas was produced erratically (that is, sometimes a bubble would form in one tube and not in the other; at other times both tubes would have a bubble, and at other times neither tube would have a bubble) in amounts never exceeding 10 per cent as measured in the Durham tube. The fermentation types recognized are illustrated in Figures 1, 2, 3, and 4.

The differences in amount and rate of gas production by the various isolates of bacteria, as expressed by the fermentation indexes, were found to be a much more useful basis for establishing the relationships between the plant pathogens and the coliform organisms than the usual qualitative interpretation of fermentation tests.

Results of the Fermentation Tests: Fermentation indexes, determined for 27 fermentable substrates, are summarized in Table 3. The cultures of *E. amylovora, E. tracheiphila,* and *E. salicis* were noteworthy for the limited range of substrates they were able to ferment with acid or gas formation. The only substrates fermented by all the isolates of those three species were glucose, fructose, sucrose, and pectin. The cultures of *E. lathyri, E. ananas,* the pectolytic organisms, the "nonpathogenic" soft-rot organisms, the corn stalk-rot bacteria, the coliform bacteria, *Serratia marcescens,* and *Proteus vulgaris,* on the other hand, produced positive reactions from a majority of the substrates tested. The tendency to ferment only a limited number of substrates indicates that *Erwinia amylovora, E. tracheiphila,* and *E. salicis* are physiologically more highly specialized than the other bacteria studied.

Not many plant pathogens were found to resemble the coliform bacteria in vigor and gas production. Only the corn stalk-rot bacteria produced fermentation reactions of the coliform type. The other plant pathogens exhibited fermentation reactions much less vigorous and are not likely, therefore, to be considered coliform bacteria. The so-called "nonpathogenic" cultures almost invariably produced coliform-like fermentation reactions.

All the isolates of *E. amylovora, E. tracheiphila,* and *E. salicis* produced acid and sometimes a small amount of gas (*E. salicis*), from glucose, fructose, and sucrose, and they failed to produce visible acid and gas from many of the other fermentable substrates commonly used in bacterial classification, such as rhamnose, maltose, cellobiose, lactose, etc.

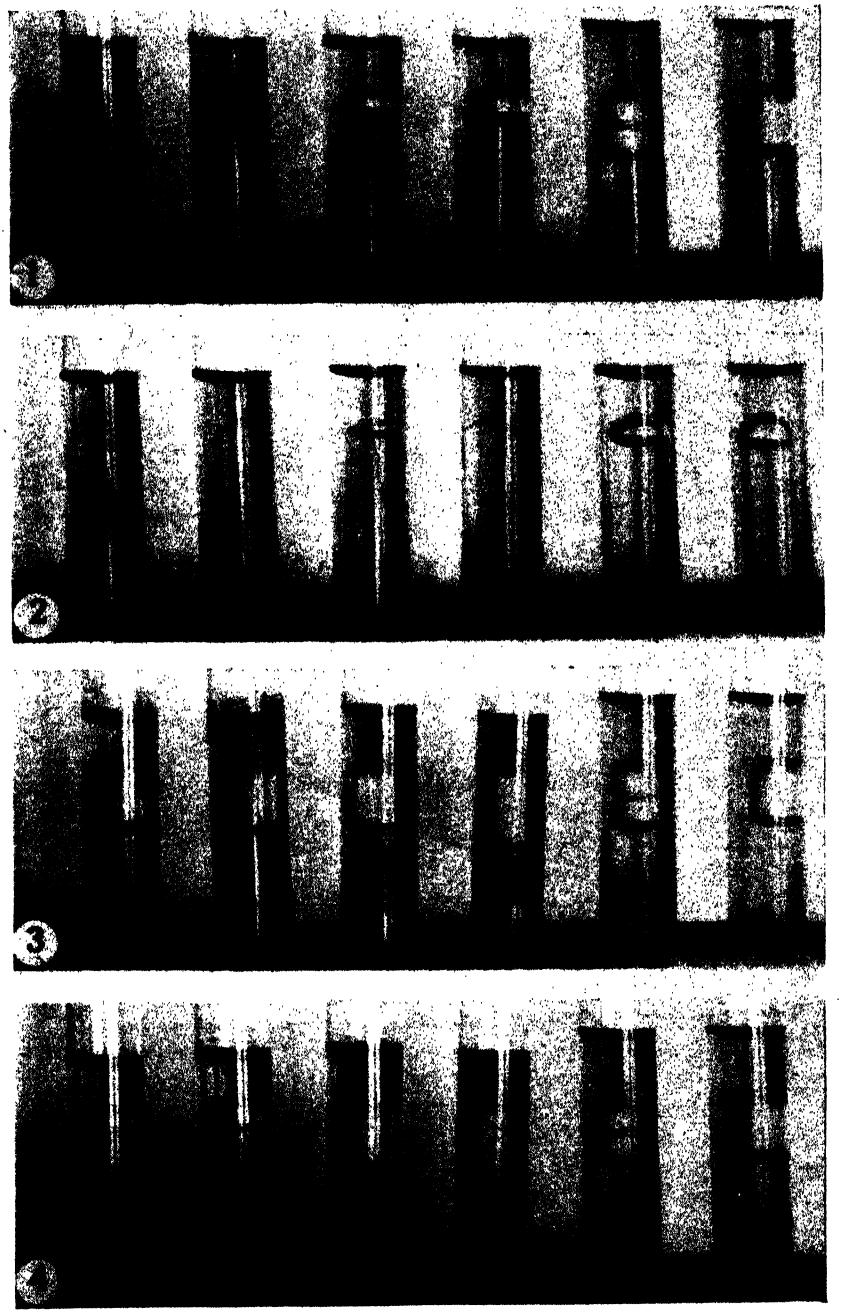
Certain fermentable substrates served to effectively differentiate these

FIG. 1. Typical fermentation reactions of *Bacillus orideae* (index 1111), *B. carotovorus* (index 2222), and *Escherichia freundii* (index 3333) in buffered peptone-sucrose broth. Arranged left to right are paired tubes of *Bacillus aroideae* (24), *B. carotovorus* (W20), and *Escherichia freundii* (204) after two weeks.

FIG. 2. Typical fermentation reactions of some gas-forming pectolytic bacteria (index 00xx) in buffered peptone-sucrose broth. The three pairs of tubes were inoculated from the same culture of *Bacillus carotovorus* (M60) and incubated for two weeks.

FIG. 3. Typical fermentation reactions of *Bacterium dissolvens* and coliform bacteria in buffered peptone-sucrose broth (index 3333). Paired tubes at the left are *Bacterium dissolvens* (W4 and W71, respectively), compared with *Escherichia freundii* on the right.

FIG. 4. Typical fermentation reactions of *Bacillus carotovorus* (index 2222), *Bacterium dissolvens* (index 3333), and *Escherichia freundii* (index 3333) in buffered peptone-sucrose broth. Paired tubes of each culture are arranged in the order named.



three species from each other. For example, by its ability to produce acid from raffinose, *E. salicis* was easily distinguished (Index 0011) from the others. The failure to produce visible acid from mannitol characterized *E. amylovora.* Likewise, the lack of visible acid formation in melibiose was characteristic of *E. tracheiphila.* Other substrates also proved to have some value for distinguishing these three species, but in some instances the production of acid was very slight.

The pectolytic bacteria differed from *E. amylovora, E. tracheiphila,* and *E. salicis* in that they were able to produce acid, or acid and gas, in measurable amounts from the pentoses and many of the oligosaccharides. The pectolytic bacteria were readily distinguished from the coliform bacteria, on the other hand, by their typical fermentation reactions of 0000 to 2222 (Figs. 1, 2, and 4), in contrast with the vigorously aerogenic reactions of the coliform bacteria (Index 3333).

With the exception of culture No. C8, all the isolates of pectolytic bacteria failed to produce visible acid or gas from maltose. These results do not substantiate the conclusions of Dowson (1940, 1941), whose studies led to the conclusion that the fermentation of maltose was an effective means of distinguishing *E. carotovora* from *E. phytophthora.* Table 3 shows that neither *E. carotovora* (No. 20) nor *E. phytophthora* (No. 23) was able to ferment maltose. In this study the maltose fermentation was, however, a valuable aid in differentiating the pectolytic organisms from the nonpathogenic organisms received as "soft-rot bacteria." The results summarized in Tables 2 and 3 show that the maltose-fermenting organisms are distinct from the pectolytic plant pathogens in many respects.

Certain strains of pectolytic bacteria were notable for their characteristically slow fermentation in most of the substrates. Culture Nos. 17, 18, M60, 21, W2, and 110 usually gave fermentation indexes of 000? to 00XX. This characteristically slow fermentation was not correlated with any other cultural, biochemical, or pathological characters. Only one culture (W2) of slow-fermenting pectolytic organisms was anaerogenic. The gas-forming strains usually produced a bubble of gas in only one of the duplicate Durham tubes inoculated (Fig. 2).

With respect to the gas-forming pectolytic organisms, the glycerol fermentation was of particular interest. In his original description, Jones (1901) described *E. carotovora* as producing a small amount of acid from glycerol. Smith (1910) pointed out that *E. phytophthora* did not produce acid from glycerol. The type culture of *E. carotovora* (No. 20) produced acid from glycerol slowly and in small amounts (Index 0001), whereas the type culture of *E. phytophthora* (No. 23) produced no visible acid from glycerol. Since the production of acid from glycerol was in all cases rather weak and slow, the differences between these two cultures may be more apparent than real. But this character was used in the original descriptions of these organisms and is even now useful in distinguishing between these two species. With respect to glycerol fermentation, six cultures (Nos. 20, 26, W20, W38, C,12, and 110) resembled *E. carotovora,*

and eight cultures (Nos. 23, V10, W120, 17, 18, M60, 21, and C8) resembled *E. phytophthora.*

All the isolates of corn stalk-rot bacteria produced fermentation reactions characteristic of the coliform bacteria. Glucose, galactose, fructose, maltose, cellobiose, and esculin were fermented vigorously by all isolates (Index 3333). With lactose, however, these organisms produced an unusual fermentation type (Index 0002 to 0033), which resembled that of the so-called slow lactose-fermenting coliform bacteria associated with certain respiratory, urinary, and intestinal infections of man (Parr, 1939). This characteristic, noted by Rosen (1926), and also evident in Stanley's (1938) data, is believed to be of major importance as a taxonomic character. In salicin the fermentation was not particularly vigorous (Index 2221). With starch and glycerol the fermentation was slow and weak (Index 0000 to 0011). Culture No. 100, however, gave an index of 0233 for starch and 3333 for glycerol, which may be interpreted to mean that this culture should be considered a separate species, in which case it must be concluded that the various isolates of the corn stalk-rot bacteria are merely slow-lactose-fermenting strains of *Aerobacter aerogenes* and *A. cloacae.* At the present time, however, it seems preferable to consider the corn stalk-rot organisms as a distinct species of coliform bacteria.

The yellow organisms *Erwinia lathyri* (Nos. 28 and 29) and *E. ananas* (No. 101) gave fermentation indexes that resembled those of *Serratia marcescens.* No gas was produced by any of the chromogenic cultures studied.

The fermentation tests show (1) that *Erwinia amylovora, E. tracheiphila,* and *E. salicis* ferment only a few of the substrates tested and that their fermentation type is characteristically anaerogenic; (2) the pectolytic bacteria ferment almost as wide a range of substrates as the coliform bacteria, but their fermentation type is much less vigorous, yielding only small amounts of gas or none; (3) several species of pectolytic bacteria can be identified by means of fermentation reactions; (4) the corn stalk-rot bacteria are readily recognized as a species of coliform bacteria; (5) the yellow bacteria exhibit fermentation reactions resembling those of *Serratia marcescens.*

It was thought that the fermentation of pure pectin would provide a convenient means for characterizing the pectolytic bacteria, but pectin was fermented by all the organisms tested. Contrary to the supposition expressed by Burkey (1928), the maceration of plant tissue was found to be due to pectopectinase activity and not to the fermentation of pectin. In these tests, no attempt was made to purify the pectin before including it with the basal medium. Elrod (1942) has done this and reported both pectolytic and coliform organisms to be capable of fermenting this material.

On the basis of their fermentation reactions (Table 3), the pectolytic bacteria may be readily divided into two groups. Twenty-three isolates of pectolytic organisms never were observed to produce gas in any of the fermentable substrates tested. The remaining 14 cultures produced gas

in small amounts (Index 00?X to 2222). Harding and Morse (1909) observed that the pectolytic organisms showed considerable variability in gas-production, and, because the amount of gas produced was in all instances scarcely more than enough to saturate the culture liquid, this variability was considered to be of little importance in the delimitation of species. The result of the present study, essentially identical with that of Harding and Morse (1909), is believed to be convincing evidence that this peculiarity in gas-production is a characteristic response of these organisms and that it is an important criterion for their classification.

TAXONOMIC GROUPING OF THE CULTURES

The difficulties in identifying and classifying the bacteria are chiefly due to lack of readily defined criteria for characterizing the taxonomic units. These problems, critically reviewed by Smith (1905), Buchanan (1929), Rahn *et al.* (1929, 1937), Kluyver and van Niel (1936), Dowson (1939), and others, have not lent themselves readily to a satisfactory solution. From the literature, one gets the impression that our ideas concerning the classification of bacteria are far in advance of our knowledge of the organisms themselves. An attempt to establish a list of criteria for use in characterizing the bacteria was made by the Society of American Bacteriologists in their Descriptive Chart. Harding (1910) pointed out that, although the classification scheme based on the chart had considerable merit, it had the serious limitation of defining bacterial species in a manner inconsistent with the ordinary conceptions of species. The extent to which these inconsistencies have resulted in a lack of system is very evident in Elliott's (1943) recent review of the classification and nomenclature of the bacterial plant pathogens.

The results of the present study provide adequate evidence to show that the species of peritrichous plant pathogens cannot be shifted *en masse* from the obsolete but convenient genus *Bacillus* of Migula (1900) to any single genus in the system of Bergey *et al.* (1923). In order that the "taxonomic anomalies" pointed out by Rahn *et al.* (1929) might be reduced to workable realities, the various species must be distributed among the genera and families in which they most obviously belong. Where suitable categories do not exist, they must be established. The results of the present study, summarized in Tables 2 and 3, suggest the following grouping of the isolates studied which seems to more adequately indicate their true relationships.

GROUP I. BACTERIA WITH LIMITED HOST RANGE AND BIOCHEMICAL ACTIVITY

Erwinia amylovora (19 isolates), *E. salicis* (3 isolates), and *E. tracheiphila* (6 isolates) are characterized by the narrow range of host plants that they are capable of attacking. Symptomatic responses on host plants may vary among the various species within the group, producing direct general or local necrosis or indirect vascular or cortical necrosis (see

Melhus and Kent, 1939). They are further characterized by the relatively large number of negative or weak biochemical activities observed in the various media used in the study, suggesting a rather high degree of physiologic specialization. The three pathogens are not particularly easy to cultivate in laboratory culture media.

The exoenzymatic activities of this group of organisms appears to be limited in scope. They do not reduce nitrates nor utilize inorganic nitrogenous compounds in their metabolism. They release no hydrogen sulphide from proteoses, nor do they produce acids in milk cultures.

They produce no indole from tryptophane. They characteristically produce a slight or indecisive acid reaction to the methyl red test. They are V-P negative and do not grow in Koser's citrate medium.

They grow slowly and relatively sparsely on nutrient agar. They grow poorly or not at all on E.M.B. and Endo-agar plates. In liquid media they grow anaerobically, as evidenced by the uniform turbidity throughout the depth of the culture tube.

The fermentation reactions of the members of this group are anaerogenic, sometimes microaerogenic, in nature. All of the species in the group produce visible fermentations in only a few of the 28 fermentable substrates tested. It may be significant that only glucose, fructose, and sucrose were fermented by all the members of the group. Galactose, mannose, melibiose, raffinose, esculin, salicin, glycerol, and mannitol were found to be useful in differentiating the various species within the group.

GROUP II. THE PECTOLYTIC BACTERIA

The pectolytic bacteria, hitherto known as the "soft-rot" bacteria, comprise a distinct group of generic rank. Included in the group are *E. carotovora* (6 isolates), *E. phytophthora* (7 isolates), *E. aroideae* (5 isolates), *E. melonis* (18 isolates), and an isolate taken from diseased delphiniums in California (Culture No. C-8). While these organisms exhibit several characteristics suggesting a possible relationship with the coliform bacteria, there is no adequate justification for concluding that they belong together in the same genus. They are distinctly different and can readily be identified by means of a few simple laboratory tests.

In all culture media the pectolytic bacteria grow much more luxuriantly than the organisms of Group I but noticeably less so than the coliform bacteria which comprise Group III. In E.M.B. and Endo-agar cultures they grow readily but produce colonies that are distinctive in appearance and smaller in size than those of the coliform organisms.

The members of Group II produce typical symptomatic responses in a wide range of fleshy host plants. They produce a rapid direct general necrosis by means of a potent protopectinase. Pigment production in the host tissues is not correlated with virulence.

The pectolytic bacteria characteristically exhibit a rapid proteolysis in gelatin media. They reduce nitrates to nitrites and utilize inorganic nitrogen in their metabolism. They release hydrogen sulphide from pro-

teoses and acidify milk cultures with curd formation but without proteolysis.

Their "IMViC" reactions tend to resemble those of *Escherichia freundii* except that they never produce indole and frequently give V-P reactions that are not easy to interpret. The V-P reaction and Koser's citrate utilization test were found to be useful in distinguishing between certain of the species.

The fermentation reactions of the pectolytic bacteria were either anaerogenic or microaerogenic in nature. They ferment a relatively wide range of fermentable substrates with acid and a small amount of gas, if any. The coliform organisms were readily distinguished from them by the vigor of their fermentation reactions in most substrates. In Durham tube cultures the pectolytic organisms never produce more than 10 per cent of gas, whereas the coliform nearly always produce more than this volume. None of the pectolytic organisms, except culture No. C-8, produced a visible fermentation reaction in maltose. Glycerol and melibiose also were found to be useful substrates for distinguishing some of the species.

The so-called "nonpathogenic" isolates do not conform to the behavior pattern herein described. They produce no protopectinase activity, produce abundant amounts of gas in most fermentable substrates, ferment lactose vigorously, and in many other respects behave in a manner characteristic of the coliform bacteria.

GROUP III. THE COLIFORM BACTERIA

The coliform bacteria, comprising Group III, are usually described as "lactose-fermenting, Gram-negative, rod-shaped bacteria which are most prevalent in the intestine and in feces, but are also common in water, milk, and soil. . . . (They are) saprophhtic but potentially pathogenic" (Malcolm, 1938). They are most readily recognized by the vigor of their fermentation reactions in media containing a fermentable carbon substrate. They produce an abundance of gas in a very short time from most substrates. The papers of Levine (1932), Malcolm (1938), Parr (1939), Rogers *et al.* (1915), and Stuart *et al.* (1938) cover in detail the characterization and significance of these organisms in water, milk, public health, soil, and disease.

Only one plant pathogen was found to rightfully belong in this group of bacteria. The corn stalk-rot bacteria exhibit a behavior pattern which definitely places them in the genus Aerobacter. Also studied were *Aerobacter aerogenes* (type culture and 7 "nonpathogenic" isolates), *A. cloacae* (type culture only), *Escherichia coli* (type culture only), and *E. freundii* (type culture and one "nonpathogenic" isolate).

The coliform bacteria do not exhibit protopectinase activity nor liquefy gelatin in the rapid manner of the pectolytic bacteria. They acidify milk cultures with curd formation but without proteolysis. They reduce nitrates to nitrites and utilize inorganic nitrogen in their metabolism. They release hydrogen sulphide from proteoses.

They grow luxuriantly in most cultures, and in E.M.B. and Endo-agar plate cultures they produce rapidly growing colonies of characteristic configuration and luster. They usually produce strong positive or negative reactions with the "IMViC" tests.

Their fermentation reactions are of the macroaerogenic type, abundant gas being produced rapidly from a wide range of fermentable substrates.

One culture (No. 496) received as *Erwinia phytophthora,* and which proved to be nonpathogenic, was the only nonpathogenic culture which was definitely a coliform organism. Its identity was not determined.

GROUP IV. THE CHROMOGENIC BACTERIA

The chromogenic bacteria differed from the other bacteria studied in a number of respects but showed many similarities with *Serratia marcescens.* They are, accordingly, considered together in Group IV. The two isolates received under the name *Erwinia lathyri* (Nos. 28 and 29) are in all probability not the same as the organism described by Manns and Taubenhaus (1913) and by Manns (1915), because in their descriptions this organism did not liquefy gelatin rapidly nor reduce nitrates, and in certain other respects behaved differently than the two cultures here studied.

The pineapple bud-rot organism was not studied very long in the course of the present study. The fact that *Erwinia ananas* does not reduce nitrates suggests that it might well belong in Group I, but the wide range of fermentable substrates attacked by it leaves its position doubtful until complete studies have been made. A number of studies with *Phytomonas stewartii* gave results which strongly suggested a rather close relationship.

CONCLUSIONS

The opinion expressed by Rahn *et al.* (1929), Stuart *et al.* (1938), Stanley (1937), and others that there is no practical means for differentiating the peritrichous plant pathogens from the coliform bacteria was found to be unwarranted. Groups I, II, and III were found to be generically distinct. It is recommended that Group I be considered to be the genus *Erwinia,* Group II be considered a new genus of bacteria, and the corn stalk-rot organism be transferred to the coliform bacteria of Group III. The chromogenic bacteria, because they are quite unlike the three above-mentioned groups and are of doubtful pathogenicity, are set aside with *Serratia marcescens,* which they seem to resemble. The so-called nonpathogenic strains of bacterial plant pathogens usually can be detected without the use of pathogenicity tests.

GENERA AND SPECIES

THE GENUS ERWINIA (WINSLOW ET AL.) EMEND.

Historical: Even before the Society of American Bacteriologists' committee on characterization and classification of bacterial types began its work, there was a strong tendency to regard *Bacillus* as comprising

only the sporogenous bacilli. In Europe the system of Lehmann and Neumann (1896) was widely followed. The ruling adopted by the Second International Microbiological Congress in London (St. John-Brooks and Breed, 1937), wherein *Bacillus* Cohn, 1873 was made a *genus conservandum* with *Bacillus subtilis* Cohn emend. Prazomowski, 1880, designated as the type species, effectively removed the peritrichous plant pathogens from consideration as members of the genus.

In the first report of the committee, Winslow *et al.* (1917) proposed that the peritrichous plant pathogens be classified together with all the other bacterial plant pathogens in the new genus *Erwinia*, which they characterized as follows with *Erwinia amylovora* designated as the type species:

> Plant pathogens. Growth usually whitish often slimy. Indol generally not produced. Acid usually formed in certain carbohydrate media, but as a rule no gas.

In their final report (1920) the genus was placed in the tribe *Erwineae* of the family *Bacteriaceae*.

Bergey *et al.* (1923) redefined the genus slightly so as to exclude all except the peritrichous plant pathogens as follows:

> Motile rods, possessing peritrichous flagella. The rods are white and a few species form pigment.

In the successive editions of the manual the generic diagnosis of *Erwinia* was emended but slightly. In the fifth edition (1939), the following description of the genus appeared:

> Plant pathogens. Invade the tissues of plants and produce local lesions, some species killing the host plants. Usually motile with peritrichous flagella. Ferment dextrose and lactose with formation of acid or acid and visible gas. Usually attack pectin.

Since its establishment, the genus has been much criticized by Rahn *et al.* (1929, 1937), Kluyver and van Neil (1936), and others on the grounds of its being characterized solely by plant pathogenicity. Dowson (1939) rejected the genus because of its being based on inadequate diagnostic characters. In the heat of discussion the fundamental concept of the genus, none too clear at best, became more and more vague. This is best illustrated by the fact that throughout the history of the genus the type species always has been *E. amylovora*. Yet in many recent papers, of which the fifth edition of Bergey's Manual (1939) and Elrod's (1942) study of the Erwinia-Coliform relationship are examples, there is a marked tendency to regard the term *Erwinia* to mean the so-called "soft-rot" group, a concept that cannot be readily reconciled with principles of sound taxonomy.

Validity of the genus Erwinia: With the principle of taxonomic types as the basis for argument, there seems to be no justification for regarding the genus *Erwinia* as invalid on the grounds of ambiguity. The results of the experimental work summarized in Tables 2 and 3 reveal two other

species studied, namely, *E. salicis* and *E. tracheiphila*, that resemble *E. amylovora* more closely than any of the other bacteria studied. Moreover, since these three organisms exhibit several important characteristics in common, which distinguish them from all other known genera of bacteria, they must be considered the nucleus of a rather sharply defined genus, and all other species, hitherto included therein but differing radically in behavior, should be classified elsewhere. It seems necessary, therefore, that the genus *Erwinia* Winslow *et al.* (1917) be emended somewhat as follows:

Heterotrophic, Gram-negative, nonspore-forming, rod-shaped bacteria. Motile by means of peritrichous flagella or nonmotile. Usually require organic nitrogen compounds for growth in laboratory culture media. Produce acid with or without small amounts of gas from a restricted number of carbon compounds in buffered peptone media. Habitat: living plants.

The type species is *E. amylovora* (Burrill) Winslow *et al.*, Jour. Bact. 2:560, 1917. Syn. *Micrococcus amylovorus* Burrill, Ill. Indust. Univ. Rept. 11:134, 1882.

KEY TO THE SPECIES

A.	Gelatin liquefied.	1. *E. amylovora*
AA.	No visible liquefaction of gelatin.	
B.	Acid from mannose, raffinose, and esculin.	2. *E. salicis*
BB.	Not as above, acid from galactose.	3. *E. tracheiphila*

CHARACTERIZATION OF THE SPECIES OF ERWINIA

1. *E. amylovora* (Burrill) Winslow *et al.*, Jour. Bact. 2:560, 1917. Syn. *Micrococcus amylovorus* Burrill, Ill. Indust. Rept. 11:134, 1882; *M. amylivorus* Burrill (typographical error), Amer. Naturalist 17:319, 1883; *Bacterium amylovorus* (Burrill) Chester, Ann. Rept. Delaware Agr. Exp. Sta. 9:127, 1897; *Bacillus amylovorus* (Burrill) Trevisan, I gen. e le spec. delle batteriaceae, p. 19, 1889; *Bacterium amylovorum* (Burrill) Chester, Manual Det. Bact., p. 176, 1901; *B. amylovorum* (Burrill) Serbinoff, Jour. Bolyzne Rostenii, No. 6:131, 1915.

Cultures No. 31, 32, 33, 34, 35, 36, 82, 83, 84, 85, 86, C-SC, C-77s, C-50, C-501, C-507, C-55, C-55.2, C-64.

Habitat: Etiological agent of the fire blight disease of pomaceous plants, producing direct general necrosis in spurs, shoots, leaves, blossoms, fruits, and cambium.

Cells are short, i.e., one and one-half to two diameters, Gram-negative, nonacid-fast, actively motile, peritrichously flagellated asporogenous bacilli with rounded ends.

In nutrient agar cultures growth is moderate, grayish-white, smooth-shiny, butyrous, and somewhat raised. In potato dextrose agar cultures growth is luxuriant but short-lived, the cultures dying out within 7 to 10 days and becoming chalky in appearance. In E.M.B. and Endo-agar plate cultures pink, pin-point colonies are formed. All peptone-containing culture fluids sustain a fairly luxuriant anaerobic growth, as evidenced by the uniform and moderate turbidity, with fairly abundant precipitate and frequently a membranous pellicle. Growth in Koser's citrate medium is nil. An alkaline reaction is produced in litmus milk cultures.

Protopectinase and amylase activity absent, but a moderately rapid proteolysis of gelatin produced. Milk sometimes peptonized.

Nitrates are not reduced nor inorganic nitrogen utilized in metabolism. Hydrogen sulphide is not released from proteoses. Indol not produced. Methyl red reaction slightly positive or indecisive. V-P reaction negative. No sustained growth in Koser's citrate medium.

Fermentation anaerogenic. Acid always produced from glucose, fructose, sucrose, and melibiose. Acid production doubtful or indecisive from arabinose, xylose, galactose, trehalose, esculin, and glycerol. Acid production definitely negative from rhamnose, sorbose, maltose, cellobiose, lactose, raffinose, dextrin, starch, cellulose, inulin, salicin, erythritol, dulcitol, mannitol, sorbitol and i-inositol.

2. *Erwinia salicis* (Day) Bergey *et al.* Man. Det. Bact. fifth ed., p. 406, 1939. Syn. *Bacterium salicis* Day, Oxford For. Mem. 3:14, 1924; *Phytomonas salicis* (Day) Magrou, Dict. Bact. Path., p. 408, 1937.

Cultures No. 79, 80, 81.

Habitat: Etiological agent of the watermark disease of *Salix* species, producing an indirect vascular necrosis and conspicuous staining of the wood.

Cells are short, i.e., one and one-half to two diameters, Gram-negative, nonacid-fast, usually actively motile, peritrichously flagellated asporogenous bacilli.

In nutrient agar cultures growth is not luxuriant, is grayish-white to translucent, spreading, smooth-shining, butyrous. In potato dextrose-agar cultures growth is more copious, somewhat more raised, and assumes a definite lemon yellow color after a few days. Pale pink, pin-point colonies are produced in E.M.B. and Endo-agar plate cultures. Luxuriant growth (anaerobic) in all peptone-containing culture solutions, as evidenced by a moderate uniform turbidity and a fairly copious precipitate but no pellicle. No visible growth in Koser's citrate medium. Litmus milk cultures remain unchanged in appearance, except that the indicator may appear very slightly reduced.

Protopectinase, amylase, and proteinase activity absent. Nitrates are not reduced, nor are inorganic nitrogenous compounds utilized in metabolism. Hydrogen sulphide is not released from proteoses. Indole not produced. Methyl red reaction slightly positive or indecisive. V-P reaction negative. Sustained growth in Koser's citrate medium negative.

Fermentation usually microaerogenic, but may be anaerogenic. Acid with slight and erratic gas production from glucose, mannose, fructose, sucrose, and mannitol. Acid only from melibiose, raffinose, esculin, and salicin. Acid production doubtful from trehalose and glycerol. Definitely negative fermentations from arabinose, xylose, rhamnose, galactose, sorbose, maltose, cellobiose, lactose, dextrin, starch, cellulose, inulin, erythritol, dulcitol, sorbitol, and i-inositol.

3. *Erwinia tracheiphila* (Smith) Holland, Jour. Bact. 5:222, 1920. Syn. *Bacillus tracheiphilus* Smith, Cent. f. Bakt. 1:364, 1895; *Bacterium tracheiphilum* (Smith), Chester, Man. Det. Bact., p. 72, 1897; *B. tracheiphilum* (Smith) Lehmann and Neumann, Vol. 2, p. 446, 1931.

Cultures No. 94, 95, 96, 97, 98, 99.

Habitat: Etiological agent of bacterial wilt disease of cucurbits, causing indirect vascular necrosis. Grayish-white exudate may be squeezed from the vascular elements of diseased plants.

Cells are relatively long, i.e., three to four diameters, Gram-negative, nonacid-fast, actively motile when young, peritrichously flagellated asporogenous bacilli.

In nutrient agar cultures, growth is grayish-white to translucent, not luxuriant, very slimy, raised, smooth-shiny, short-lived. In potato dextrose-agar cultures, growth is much more luxuriant and longer lived, the translucent appearance predominating. No growth in E.M.B. or Endo-agar plate cultures. A very faint turbidity without precipitate or pellicle formation in all peptone-containing culture fluids. No visible growth in Koser's citrate medium. Litmus milk cultures remain unchanged in appearance.

Protopectinase, amylase, and proteinase activity lacking in substrates used. Nitrates not reduced, and inorganic nitrogen not utilized in metabolism. Hydrogen sulphide not released from proteoses. Indole is not produced. Methyl red reaction slightly positive or indecisive. V-P reaction negative. Koser's citrate utilization test negative.

Fermentation anaerogenic. Acids produced from glucose, galactose, fructose, sucrose, and mannitol. Acid production negative from arabinose, xylose, rhamnose, mannose, sorbose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, dextrin, starch, cellulose, inulin, esculin, glycerol, erythritol, dulcitol, sorbitol, and i-inositol.

TAXONOMIC POSITION OF THE GENUS ERWINIA

With the emendation of the genus Erwinia herein proposed, there arises the problem of which family to put it in. That it cannot be included in the family *Enterobacteriaceae* of Bergey's Manual (1939) is evident from the fact that the genus cannot be keyed into this family or even the tribe *Erwineae*, of which it is the type genus. The species included in the emended genus do not reduce nitrates, ferment lactose, release hydrogen sulphide from proteoses, nor excrete protopectinase, and they ferment carbohydrates anaerogenically or, rarely, microaerogenically. Inasmuch as the family *Enterobacteriaceae* was specifically defined to include the coliform bacteria and closely related genera, it cannot accommodate the genus *Erwinia*, unless the family diagnosis be drastically emended. It would seem preferable to establish a new taxonomic unit of family rank, perhaps, to include those genera of physiologically specialized bacteria resembling the emended *Erwinia*. A new family bearing the name *Erwiniaceae fam. nov.* with the genus *Erwinia* (Winslow *et al.*) 1917 *emend.* designated as the type, is characterized as follows:

Heterotrophic, Gram-negative, asporogenous bacilli. Exhibit considerable physiological specialization. Usually require a complex source of nitrogen and/or growth factors. Do not reduce nitrates. Acid fermentation from a relatively few fermentable substrates with or without small amounts of gas. Habitat diverse.

The type genus is *Erwinia* (Winslow *et al.*, 1917) *emend.* This designation of the family would exclude the pectolytic and all other bacteria that do not conform to the behavior pattern outlined above. Many species now included in the general Flavobacterium, Fusobacterium, Bacteroides, Actinobacillus, Achromobacter, Phytomonas, and Bacterium may be found to be rightfully classified in this same taxonomic unit. The establishment of a family such as this is justified not only from taxonomic necessity but also by the criteria already established by Buchanan (1917-18), which have since become the basis of modern bacterial taxonomy in this country.

THE GENUS **PECTOBACTERIUM** GEN. NOV.

Historical. Although the soft-rot diseases of plants have been under investigation for more than half a century, innumerable problems regarding the identities of the various etiological agents remain unsolved. Heinz's (1889) investigation of the white rot disease of the hyacinth was the first accurate study of an undoubted soft-rot disease of plants. His description of the causal organism, which he called *Bacillus hyacinthi septicus*, was sketchy, and, because of this, together with the fact that his epithet is a trinomial, his name cannot now be considered valid. Most of the early studies of the soft-rot and blackleg organisms were likewise inadequate for accurate confirmation and thus have provided the basis for nomenclatural controversies, some of which have never been settled satisfactorily.

Potter's (1901) investigations into the nature of the white rot disease of turnip, followed shortly by Jones' (1901, 1909) monumental studies of the carrot rot disease, both proved the pectolytic nature of the patho-

genesis of these pathogens, and are classics in the annals of phyto-pathological research.

The identity of Potter's organism soon became a matter of doubt, however, because Potter (1901) reported polar flagella for his species, *Pseudomonas destructans*. Unfortunately, Potter's original culture was lost, and subsequent isolates sent by him to Harding and Morse (1909) proved to be peritrichous. More recently, S. G. Jones (1922) described a bacterium with polar flagella isolated from a soft-rot of turnip which he thought might be the same as *Pseudomonas destructans* Potter (1901). Because Jones' isolate differed in several important respects from the soft-rot organisms under consideration, the validity of Potter's epithet became more doubtful than ever, and should, therefore, probably be considered a *nomen confusum*. The earliest valid, adequately described species of peritrichous vegetable soft-rot bacteria is, therefore, *Bacillus carotovorus* Jones (1901).

The bacterial nature of the potato blackleg disease was first definitely proved by Frank (1898), who described and named the pathogen. His organism, *Micrococcus phytophthorus*, was not adequately described, for which reason Appel (1902) declared the epithet invalid and proposed the name *Bacillus phytophthorus* Appel. A few months later van Hall (1902) published a complete description of the potato blackleg pathogen, which he called *B. atrosepticus*. Appel's (1903) detailed description of the pathogen did not appear until the following year. Whether Appel's or van Hall's name should be accepted has been a controversial point to this day. That Frank was dealing with the blackleg pathogen cannot be doubted from his reported positive inoculation results. Furthermore, his description of the isolated organism indicates the presence of morphologic forms fundamentally characteristic of the species. A careful perusal of Frank's papers reveals that he observed very short rod-shaped organisms in his cultures which he regarded as aberrant forms. In view of his observations, if not his interpretation of them, Frank's epithet must be considered valid, despite the widely held opinion that Appel or van Hall should be accorded the credit. Thus *Micrococcus phytophthorus* Frank (1899) appears to be the first valid epithet that can be applied to a member of the pectolytic bacteria.

During the years following these early studies, several species of soft-rot bacteria were described, some of which are readily distinguishable species, but most of which are merely synonyms of earlier published epithets. Townsend's (1904) *Bacillus aroideae* and Gidding's (1910) *B. melonis* can be considered valid species. The others, for the most part, can be readily recognized as synonyms of one of the four species already mentioned.

Because of the close similarities existing among the various species of pectolytic bacteria, comparative studies with them have been numerous, and many conflicting conclusions have been derived from them. The earliest comparative study of note was by Harding and Morse (1909), whose observations of 43 strains of soft-rot bacteria isolated from several

species of plants in scattered parts of the world, led them to conclude that *B. carotovorus, B. omnivorus, B. oleraceae, B, aroideae,* and "Potter's bacillus" were practically indistinguishable, and that they should be considered as somewhat variant strains of a single species. Although they noted a strong tendency for many of the isolates to produce small amounts of gas erratically, they commented on the remarkable stability and uniformity exhibited by the organisms with respect to all other cultural characters. Their careful observations provide a reliable basis today for characterizing the pectolytic bacteria. Their data show that some of their cultures were never observed to produce gas, a fact that has been confirmed in the present study, and the fundamental importance of which apparently has been overlooked.

On the basis of differences in fermentative ability, cultural characters and pathogenicity among *B. carotovorus, B. aroideae,* and an isolate from tomato, Massey (1924) concluded that the organisms, though closely related, should be considered two separate species. He also reported the observation that *B. phytophthorus* and *B. atrosepticus* differed from *B. carotovorus* in their ability to produce acid in ethyl alcohol media. He suggested, too, that the failure of the soft-rot bacteria to produce acid or gas from maltose may be due to the absence of the alphaglucosidase, maltase, in their enzymatic complex.

Massey's (1924) conclusions were supported by the cross agglutination studies of Link and Taliaferro (1928). Matsumoto (1929) and Matsumoto and Samazowi (1931), also using serological methods, obtained clear differences among strains of soft-rot bacteria that appeared identical in other respects. That *B. aroideae* is a distinct species from *B. carotovorus* was further supported by pathological, physiological, and serological evidence reported by Brierley (1928), Leach (1930), Johnson and Valleau (1931), Dowson (1939, 1941), and Elrod (1941).

Baldacci (1934) concluded from a comparative study of several strains of soft-rot bacteria, that *B. apivorus* Wormald (1913) and *Bacterium apii* Brizi (1896) should be regarded as synonyms of *Bacillus carotovorus* Jones (1901). Because the original description of *Bacterium apii* is so meager that it is now impossible to establish its identity, it is probably more appropriate to regard it as a *nomen dubium* than a synonym.

Much confusion exists in the literature with reference to the blackleg pathogens. Data obtained from pathological, morphological, cultural, biochemical, and serological techniques led Paine and Chaudhuri (1923), St. John-Brooks *et al.* (1925) Mehta (1925), Lacey (1926), and Berridge (1926) to conclude that *Bacillus phytophthorus* (*B. atrosepticus*) was distinct from *B. solanisaprus.* From the evidence presented in these papers, there is reason to believe that *B. solanisaprus* is very similar to, if not identical with, *B. carotovorus* in all important characters except, perhaps, its virulence on potato stems. The detailed serological evidence presented by Stapp (1928) also strongly supported this view. The present cross-inoculations and laboratory studies confirm the conclusion that *B. solani-*

saprus should be considered as a synonym of *Erwinia carotovora* and not of *E. phytophthora*.

Perhaps the most comprehensive comparative study ever made on a group of bacterial plant pathogens was reported by Stapp (1928) on 128 isolates of blackleg and other soft-rot bacteria. Although he was unable to effect a satisfactory species separation by means of the usual morphological, cultural, and physiological tests, he was able to recognize five distinct and constant serological groups. While he was willing to recognize only one species, he pointed out that there may be justification for recognizing two. Leach (1930) and Bonde (1939) have confirmed the general conclusions of Stapp (1928). The nomenclature controversy arising from Stapp's (1935) acceptance of the principle of the most representative type and Leach's (1930) adherence to the principle of priority probably is most readily resolved by recognizing at least two species of organisms, namely, *Bacillus phytophthorus* Appel (1902) for the blackleg pathogen and *B. carotovorus* Jones (1901) for the pectolytic bacteria resembling Jones' isolate.

Stanley (1938) concluded, from an extensive comparative study of 120 strains of soft-rot, corn stalk-rot, and coliform bacteria that no adequate classification of these organisms is possible because of the great variability and instability reported by him.

Matsumoto and Sawada (1938) described a bacteriophage isolated from a culture of *B. aroideae* which they reported to be highly specific. Even in high concentrations the phage failed to produce visible lysis in any soft-rot culture except in cultures of *B. aroideae*.

Dowson's (1941) paper on the soft-rot bacteria has extended and confirmed the conclusions of the earlier British workers. He found maltose, xylose, gelatin, and sucrose to be useful materials in distinguishing between *Bacterium carotovorum, B. phytophthorum, B. aroideae, B. rhaponticum, B. aerogenes, Pseudomonas marginalis,* and *Ps. syringae*. This work seems to have been confirmed in recent data presented by Elrod (1941, 1942).

Serologically Elrod found *Erwinia amylovora, E. salicis, E. tracheiphila,* and the soft-rot organisms to be distinct from each other. Within soft-rot bacteria, however, he obtained two distinct serological groups, which he observed to be correlated with the ability to ferment maltose. The biochemical data reported in his paper strongly indicate that the maltose-fermenting strains were undoubtedly members of the coliform bacteria rather than true soft-rot organisms. Elrod (1942) believed that although the reactions of the soft-rot bacteria were similar to those of *Escherichia freundii* and *Aerobacter cloacae,* these organisms should be classified in a separate genus (*Erwinia*).

Classification of the pectolytic bacteria. The oft-repeated contention that the variability of the "soft-rot" bacteria is such as to make them indistinguishable from the coliform bacteria was found to be unjustified in this study. The only serious source of variation noted was in gas-production, a phenomenon which was reported by Harding and Morse (1909)

and many others and which must now be considered a distinguishing characteristic for practically all the aerogenic members of the pectolytic bacteria. No anaerogenic strain was ever observed to produce gas, and all aerogenic strains produced gas erratically and in small amounts.

The results of the experiments, summarized in Tables 2 and 3, show the pectolytic bacteria to be totally unrelated to the members of the emended genus *Erwinia*, and, although exhibiting some rather striking relationships to the coliform bacteria and *Serratia marcescens*, there can be no doubt that they are distinctive enough to be classified in a separate genus.

Since the results of this study show that the pectolytic bacteria are clearly distinct from the Erwinia species on the one hand and the coliform bacteria on the other and thus constitute a distinct, easily recognized taxonomic unit, this group of organisms should be recognized as a separate genus in the family *Enterobacteriaceae*, the diagnosis of which is as follows:

Pectobacterium n. gen.

Non-spore forming, gram-negative, rod-shaped bacteria, motile by means of peritrichous flagella or nonmotile. Grow readily in media containing inorganic nitrogen compounds and appropriate carbon source. Reduce nitrates to nitrites. In buffered peptone media produce anaerogenic or microaerogenic acid fermentation (gas none to 10 per cent in Durham tubes) from a wide range of substrates. Secrete an active protopectinase capable of producing a visible softening of raw carrot (or other fleshy plant tissue) within 48 hours or less. Active gelatinase produced. Litmus milk acidified without digestion.

The type species is *Pectobacterium carotovorum* (Jones) n. comb. Syn. *Bacillus carotovorus* Jones, Zentrbl. f. Bakt. 7:12. 1901.

KEY TO THE SPECIES

A. Gas usually formed in peptone-carbohydrate media (e.g., glucose, sucrose, lactose), in amounts less than 10 per cent in Durham tube cultures.

B. Good growth in Koser's citrate medium

C. Acid produced from glycerol slowly and in small amounts. 1. *P. carotovorum*

CC. No visible acid from glycerol 2. *P. phytophthorum*

BB. No visible growth in Koser's citrate medium 3. *P. delphinii*

AA. Gas never[1] formed in peptone-carbohydrate media in Durham tube cultures.

BB. Voges-Proskauer test negative 4. *P. aroideae*

B. Voges-Proskauer test positive 5. *P. melonis*

CHARACTERIZATION OF THE SPECIES OF PECTOBACTERIUM

1. *P. carotovorum* (Jones) *comb. nov.* Syn. *Bacillus carotovorus* Jones, Cent. f. Bakt. (II) 7:12, 1901; *Erwinia carotovora* (Jones) Holland, Jour. Bact. 5:222, 1920; *Bacterium carotovorum* (Jones) K. B. Lehmann, in Lehm., Neum. and Breed 2:446,

[1] Duplicate Durham tubes, with 1 per cent peptone, 0.1 per cent K_2HPO_4, and 0.5 to 1.0 per cent each of glucose, sucrose, and lactose repeated at least once, yielding no tubes with gas bubbles are construed as being anaerogenic.

1931; *Bacillus oleraceae* Harrison, Science (n. s.) 16:152, 1902; *Erwinia oleraceae* (Harrison) Holland, Jour. Bact. 5:222, 1920; *Bacillus omnivorus* van Hall, PhD diss. Univ. Amsterdam, p. 123, 1902; *B. solanisaprus* Harrison, Cent. f. Bact. (II) 17:34, 1906; *Erwinia solanisapra* (Harrison) Holland, Jour. Bact. 5:222, 1920; *Bacillus apiovorous* Wormald, Jour. S. E. Agr. College Wye, No. 22:457,1913;

Probable syn. *Bacillus cepivorus* Delacroix, Ann. Inst. Nat. Agron. (Ser. 2) 5:368, 1906; *Bacterium cepivorus* (Delacroix) Stapp, in Sorauer's Handbuch d. Pflanzenkr. 2 (5th ed.) 49, 1928; *Aplanobacter cepivorus* (Delacroix) Elliott, Man. Bact. Plant Pathogens, p. 4, 1930; *Bacillus cypripedii* Hori, Cent. f. Bakt. 31:85, 1912; *Erwinia cypripedii* (Hori) Bergey *et al.*, Man. Det. Bact., p. 171, 1923; *Bacillus betivorus* Takimoto, Ann. Phytopath. Soc. Japan 2:350, 1931; *Erwinia betivora* (Takimoto) Magrou, in Dict. Bact. Path., p. 200, 1937; *Bacillus sachari* Roldan, Philippine Agr. 20:259, 1931; *Erwinia sachari* Roldan, *ibid*, 1931.

Invalid. *Bacillus hyacinthi septicus* Heinz, Cent. f. Bakt. 5:535, 1889, is a trinomial, of which the following names are declared to be synonyms: *Bacterium hyacinthi septicus* (Heinz) Chester, Ann. Rept. Del. Agr. Exp. Sta. 9:127, 1897; *Bacillus hyacinthi* (Heinz) Migula, Syst. Bakt. 2:874, 1900. *Pseudomonas destructans* Potter, Proc. Univ. Durham Phil. Soc., p. 3, 1899, is a *nomen confusum*, of which the following names are declared to be synonyms: *Bacterium destructans* (Potter) Nataka *et al.*, Tech. Rep. Korea Industr. Model Farm 6:1, 1922; *Phytomonas destructans* (Potter) Bergey *et al.*, Man. Det. Bact., 3rd ed., p. 264, 1930. *Bacillus brassicaevorus* Delacroix, Comptes Rend. Acad. Sci. (Paris) 140:1356, 1905, is a green fluorescent organism which was found by Szymanek (Rev. Path. Veg. et Ent. Agr. 13:259, 1926) to be a saprophyte following larval attacks of *Contarinia torquens*.

Cultures No. 20, 26, C12, W20, W38, 110.

Habitat: Etiological agent for soft-rot diseases of a wide range of fleshy host plants, causing a rapid direct general necrosis by means of the secretion of a potent protopectinase. The maceration of host tissues usually is not accompanied by blackening of the tissues.

Cells are short, i.e., one and one-half to three diameters, Gram-negative, nonacid-fast, usually actively motile, peritrichously flagellated, asporogenous bacilli.

In nutrient-agar cultures, growth is moderately luxuriant, grayish white to cream-colored, raised, smooth shining to dull, butyrous. In potato dextrose-agar cultures, growth is much more copious and usually inclined to a creamish color. In E.M.B. and Endo-agar plate cultures, colonies usually are small, 1 to 3 mm. in diameter, with a metallic sheen resembling that of *Escherichia* species, but general appearance of colonies is quite unlike that of coliform organisms. Luxuriant anaerobic growth in all peptone-containing culture fluids, pellicle and precipitate formation varying. Growth in Koser's citrate medium luxuriant. Litmus milk cultures quickly acidified with subsequent curd formation and reduction of indicator.

Protopectinase activity strong in various living plant tissues, amylase activity lacking, proteinase activity strong in gelatin cultures but absent in milk. Nitrates are reduced to nitrites, various inorganic nitrogenous compounds being utilized in metabolism. Hydrogen sulphide released from proteoses in moderate amounts. Indole not produced. Methyl red reaction moderately to strongly positive. V-P reaction negative. Vigorous growth in Koser's citrate medium.

Fermentation reactions microaerogenic or anaerogenic in some substrates. Some strains exhibit conspicuously slow fermentation reactions, definite reactions appearing only after a week's incubation. Acids, with slight and erratic gas production by all strains from arabinose, xylose, rhamnose, galactose, mannose, cellobiose, lactose, sucrose, raffinose, and mannitol. Slow-fermenting strains produce acid only from glucose, fructose, trehalose, melibiose, and esculin, whereas other strains produce both acid and gas. All strains produce acid only from salicin and glycerol (glycerol reaction always slow). Fermentation reactions decidedly negative with sorbose, maltose, dextrin, starch, cellulose, inulin, erythritol, dulcitol, sorbitol, and i-inositol.

Salient distinguishing characters of the species are its microaerogenesis, in fermentable substrates, slow acid production from glycerol, inability to ferment maltose, ability to grow in Koser's citrate medium, methyl red-positive and V-P-negative reactions. Usually produces soft-rot without pigment fermentation in plant tissues.

2. *Pectobacterium phytophthorum* (Frank) *comb. nov.* Syn. *Micrococcus phytophthorus* Frank, Bericht. d. deut. bot. Gesellsch. 16:277, 1898; *Bacillus phytophthorus* Appel, Bericht. d. deutsch. bot. Gesellsch. 20:129, 1902; *B. phytophthorus* (Frank) Appel, in Delacroix, G., Ann. l' Inst. Nat. Agron. 5:360, 1906; *Erwinia phytophthora* (Appel) Holland, Jour. Bact. 5:222, 1920; *Bacterium phytophthorum* (Appel) Stapp, in Lehmann, Neumann & Breed, 2:446, 1931; *Bacilus atrosepticus* van Hall, Inaug. Diss. Univ. Amsterdam, p. 134, 1902; *Erwinia atroseptica* (van Hall) Bergey *et al.*, Man. Det. Bact., p. 172, 1923; *Bacillus melanogenes* Peth. & Murphy, Jour. Dept. Agr. & Tech. Inst. Ireland 10:251, 1910.

Cultures: Nos. 23, 17, 18, 21, M60, C10, W120.

Habitat: Etiological agent of the blackleg and soft-rot disease of the potato, causing a rapid direct general necrosis effected by the secretion of a potent protopectinase. Maceration of host tissue is frequently accompanied by blackening of infected tissue.

Cells are short to moderately long, i.e., one and one-half to four diameters, Gram-negative, nonacid-fast, usually actively motile, peritrichously flagellated, asporogenous bacilli with rounded ends.

In nutrient-agar cultures, growth is moderately luxuriant, grayish-white to cream-colored, smooth shining to dull, butyrous. Potato dextrose-agar cultures produce copious growth inclined to be cream-colored. In E.M.B. and Endo-agar plate cultures, colonies are usually small, 1 to 3 mm. in diameter, with a metallic sheen resembling that of Escherichia species, but general appearance of colonies quite unlike that of coliform bacteria. Luxuriant anaerobic growth in all peptone-containing culture fluids, pellicle and precipitate varying in amount. Growth in Koser's citrate medium luxuriant. Litmus milk cultures quickly acidified with subsequent curd formation and reduction of indicator.

Protopectinase activity moderately strong in various living plant tissues, amylase activity lacking, proteinase activity strong in gelatin but absent in milk. Nitrates reduced to nitrites, various inorganic nitrogenous compounds being utilized in metabolism. Hydrogen sulphide released from proteoses in moderate amounts. Indole not produced. Methyl red reaction moderately to strongly positive. V-P reaction negative. Koser's citrate test positive.

Fermentation reactions microaerogenic or anaerogenic in some substrates. Some strains exhibit conspicuously slow fermentation reactions, definite reactions not apparent until a week after inoculation. Acids, with slight and erratic gas production, produced by all strains from arabinose, xylose, rhamnose, glucose, mannose, fructose, lactose, sucrose, raffinose, and mannitol. Slow-fermenting strains produced acid only from galactose, cellobiose, trehalose, esculin, and salicin, whereas the other strains produce both acid and gas. All strains produce acid anaerogenically from melibiose. Fermentation reactions decidedly negative with sorbose, maltose, dextrin, starch, cellulose, inulin, glycerol, erythritol, dulcitol, sorbitol, and i-inositol.

Salient distinguishing characters of the species are its microaerogenesis in fermentable substrates, inability to ferment maltose and glycerol, ability to grow in Koser's citrate medium, methyl red positive and V-P negative. Produces soft-rot of potato stems and tubers frequently with formation of black pigment in the tissues.

3. **Pectobacterium delphinii** spec. nov. Type culture, C8, isolated in 1936 from blight disease of *Delphinium* spp. by P. A. Ark, in California (Phytopath. 28:281-283. 1938).

Cultures: C8.

Habitat: Etiological agent of larkspur bacterial blight, causing a rapid direct general necrosis by means of a potent protopectinase.

Cells are short, i.e., one and one-half to two diameters, asporogenous, Gram-negative, nonacid-fast, actively motile, peritrichously flagellated bacilli with rounded ends.

In nutrient-agar cultures, growth is moderately luxuriant, grayish-white to cream-colored, raised, smooth shining to dull, butyrous. Potato dextrose agar cultures produce copious growth inclined to be cream-colored. In E.M.B. and Endo-agar plate cultures, colonies are usually small, 1 to 3 mm. in diameter, with a metallic sheen resembling that produced by Escherichia species, but general appearance of

colonies quite unlike that of any coliform bacteria. Luxuriant anaerobic growth in all peptone-containing culture fluids, pellicle and precipitate varying in amount. Does not grow in Koser's citrate medium. Litmus milk cultures quickly acidified with subsequent curd formation and reduction of indicator.

Protopectinase activity moderately strong in various living plant tissues, amylase activity lacking, proteinase activity strong in gelatin but absent in milk. Nitrates reduced to nitrites, various inorganic nitrogenous compounds being utilized in metabolism. Hydrogen sulphide released from proteoses in moderate amounts. Indole not produced. Methyl red reaction positive. V-P reaction negative. Koser citrate test negative.

Fermentation reactions microaerogenic, or anaerogenic in some substrates. In general, fermentation reactions resemble those of *P. phytophthorum*. Microaerogenic acid fermentation reactions from glucose, galactose, cellobiose, trehalose, lactose, and sucrose. Anaerogenic acid fermentation reactions from fructose, maltose, raffinose (very weak to doubtful), esculin, and salicin. Definitely negative reactions with melibiose, dextrin, starch, cellulose, and glycerol. Tests were not made with arabinose, xylose, rhamnose, mannose, sorbose, inulin, erythritol, dulcitol, mannitol, sorbitol, and i-inositol.

Salient distinguishing characters of the species are its microaerogenesis in many fermentable substrates, inability to ferment glycerol, slow and rather weak fermentation of maltose, inability to grow in Koser's citrate medium, methyl red positive reaction and V-P negative reaction.

4. *P. aroideae* (Townsend) *comb. nov.* Syn. *Bacillus aroideae* Townsend, U. S. Dept. Agr. B.P.I. Bul. 60, p. 40, 1904; *Erwinia aroideae* (Townsend) Holland, Jour. Bact. 5:222, 1920; *Bacterium aroideae* (Townsend) Stapp, Sorauer's Handbuch d. Pflanzenker. 2(5):41, 1928; *Erwinia aroidea* (Towns.) Holland in Bergey *et al.*, Man. Det. Bact., p. 171, 1923; *Bacillus aroideus* (Towns.) Holland, *ibid*, 1923; *B. papaveris* Ram Ayyar, Mem. Dept. Agr. India, Bact. Ser. 2:33, 1927; *Erwinia papaveris* (Ram Ayyar) Magrou, in Dict. Bact. Path., p. 214, 1937; *E. cytolytica* Chester, Phytopath. 28:427, 1938.

Probable syn. *Bacillus croci* Mizusawa, Kanag. Agr. Exp. Sta. Bul. No. 51:1, 1921; *Erwinia croci* (Miz.) Magrou, in Dict. Bact. Path., p. 204, 1937; *Bacillus cacticidus* Johnson & Hitchcock, Trans. & Proc. Roy. Soc. S. Australia, 47:162, 1923; *Erwinia cacticida* (J. & H.) Magrou, in Dict. Bact. Path., p. 201, 1937.

Cultures: Nos. 24, 27, M10, M81, W1.

Habitat: Etiological agent for soft-rot diseases of a wide range of fleshy ornamental and economic plants, causing a rapid direct general necrosis by means of a potent protopectinase. The virulence of this species is usually much higher than the microaerogenic pectolytic bacteria.

Cells are short, i.e., one and one-half to three diameters, asporogenous, Gram-negative, nonacid-fast, usually actively motile, peritrichously flagellated bacilli with rounded ends.

Growth in nutrient agar cultures is moderately luxuriant, grayish-white to cream-colored, raised, smooth-shining to dull, butyrous. In potato dextrose-agar cultures, growth is much more copious and inclined to be a cream color. In E.M.B. and Endo-agar plate cultures, colonies usually are small, 1 to 3 mm. in diameter, with an Escherichia-like sheen, but in general appearance quite unlike those of any coliform bacteria. Luxuriant anaerobic growth in all peptone-containing culture fluids, pellicle and precipitate formation mostly quite abundant in amount. Growth in Koser's citrate medium luxuriant. Litmus milk cultures quickly acidified with subsequent curd formation and reduction of the indicator.

Protopectinase activity usually very strong in various living plant tissues. Amylase activity lacking. Proteinase activity strong in gelatin but lacking in milk. Nitrates reduced to nitrites, various inorganic nitrogenous compounds being utilized in metabolism. Hydrogen sulphide released from proteoses in moderate amounts. Indole not produced. Methyl red reaction slightly positive to indecisive or negative. V-P test usually strongly positive. Koser's citrate test positive.

Fermentation reactions are always anaerogenic in all fermentable substrates.

Acid reactions produced from arabinose, xylose, rhamnose, glucose, galactose, mannose, fructose, cellobiose, trehalose, lactose, melibiose, sucrose, raffinose, esculin, salicin, glycerol (weak), and mannitol. Definitely negative fermentation reactions from sorbose, maltose, dextrin, starch, cellulose, inulin, erythritol, dulcitol, sorbitol, and i-inositol.

Salient distinguishing characters for the species are its motility, anaerogenesis in all fermentable substrates, doubtful methyl red reaction, positive V-P reaction, and its usually very high virulence.

5. *Pectobacterium melonis* (Giddings) *comb. nov.* Syn. *Bacillus melonis* Giddings, Vt. Agr. Exp. Sta. Bul. No. 148, 1910; *Erwinia melonis* (Giddings) Holland, Jour. Bact. 5:222, 1920.

Cultures: Nos. 25, 19, M12, 22, 75, K1, K2, C11, W2, W3, W13, W42, 106, 107, 108, 109, 111, 112.

Habitat: Etiological agent for the bacterial soft-rot diseases of several species of fleshy economic and ornamental plants causing a rapid direct general necrosis by means of a potent protopectinase. Isolates of this species characteristically have a very high virulence.

Cells are short, one and one-half to three diameters, asporogenous, Gram-negative, nonacid-fast, usually actively motile, peritrichously flagellated bacilli with rounded ends.

Growth in nutrient-agar cultures is moderately luxuriant, grayish-white to creamish, raised, smooth shining to dull, butyrous. In potato dextrose-agar cultures, growth is much more copious and inclined to be cream colored. In E.M.B. and Endo-agar plate cultures, colonies are small, 1 to 3 mm. in diameter, with a metallic Escherichia-like sheen, but in general appearance the colonies are quite unlike that of any coliform bacteria. Luxuriant anaerobic growth in all peptone-containing culture fluids, pellicle formation varying but usually with a heavy precipitate. Luxuriant growth in Koser's citrate medium. Litmus milk cultures quickly acidified with subsequent curd formation and reduction of indicator.

Protopectinase activity usually very strong in various living plant tissues. Amylase activity lacking. Proteinase activity strong in gelatin cultures but lacking in milk. Nitrates reduced to nitrites, various inorganic nitrogenous compounds readily utilized in metabolism. Hydrogen sulphide released from proteoses in moderate amounts. Indole not produced. Methyl red reaction moderately to strongly positive. V-P reaction negative. Koser's citrate test strongly positive.

Fermentation reactions are always anaerogenic in all fermentable substrates. Occasional isolates produce very slow and weak acid reactions in most substrates. The weakly fermenting strains produce slight to moderate acid reactions after 1 week's incubation from glucose, galactose, fructose, lactose, melibiose, sucrose, raffinose, esculin, and salicin. They do not ferment maltose, cellobiose, trehalose, starch, cellulose, and glycerol. Reactions with other substrates were not determined. All other isolates produced prompt acid reactions from arabinose, xylose, rhamnose, glucose, galactose, mannose, fructose, cellobiose, trehalose, lactose, melibiose, sucrose, raffinose, esculin, salicin, glycerol (weak), and mannitol. Definitely negative fermentation reactions with sorbose, maltose, dextrin, starch, cellulose, inulin, erythritol, dulcitol, sorbitol, and i-inositol.

Salient distinguishing characters for the species are its anaerogenesis in all fermentable substrates, positive methyl red reaction, negative V-P reaction, and its usually very high virulence.

AEROBACTER DISSOLVENS (ROSEN) COMB. NOV. AND OTHER COLIFORM BACTERIA

The corn stalk-rot organism described by Rosen (1922) under the name *Pseudomonas dissolvens* (*Bacterium dissolven, Phytomonas dissolvens*) was among the organisms included in this study. Rosen (1926)

noted that this organism was closely related to *Bacillus coli*. Stanley (1938) considered the corn stalk-rot bacteria and soft-rot bacteria to be strains of the colon-typhoid-dysentery group. Chester in the fifth edition of Bergey's Manual (1939) regarded the corn stalk-rot organism (*Pseudomonas dissolvens*) as a possible synonym of *Erwinia carotovora*.

The results of the comparative studies summarized in Tables 2 and 3 definitely show that the corn stalk-rot organisms are coliform bacteria and do not belong to the soft-rot or pectolytic bacteria.

The corn-stalk-rot bacteria differed from the pectolytic bacteria in that they did not produce detectable protopectinase activity or gelatinase activity of any consequence. They produced "IMViC" reactions of the Aerobacter type and fermented carbohydrates, glucosides, and alcohols with abundant gas (10 to 100 per cent). They always produced acid and gas from maltose.

They resembled the species of Aerobacter in all respects except that they produced a strikingly slow microaerogenic fermentation from lactose. In lactose the fermentation index of the corn stalk-rot organism was usually 00X3, the total amount of gas generally fluctuating slightly above and below 10 per cent in Durham tubes. These observations are borne out in the data published by Rosen (1926), Stanley (1938), and Elrod (1941, 1942).

The evidence presented here and that found in the literature adequately justifies the inclusion of this species in the genus Aerobacter Beijerinck, 1900.

Characterization of the species: Aerobacter dissolvens (Rosen) *Comb. Nov. Syn.*. *Pseudomonas dissolvens* Rosen, Phytopath. 12:497, 1922; *Phytomonas dissolvens* (Rosen) Rosen, *ibid.* 16:241, 1926; *Bacterium dissolvens* (Rosen) Rosen, *ibid.*, 1926; *Aplanobacter dissolvens* (Rosen) Rosen, *ibid.*, 1926.

Habitat: Etiological agent of the corn stalk-rot disease.

Detailed description of the species was published by H. R. Rosen in Phytopath. 16:241-267, 1926; also in Arkansas Agr. Exp. Sta. Bul. No. 209, 1926.

Salient distinguishing characters of *Aerobacter dissolvens* are as follows: Gram-negative, asporogenous, plump bacilli with rounded ends. Produce abundant growth in all culture media. Produce cracks in potato dextrose-agar by virtue of its vigorous gas-production. In litmus milk cultures produce acid with curd and reduction of indicator. Produces large Aerobacter-like colonies in E.M.B. and Endo-agar plate cultures. Reduce nitrates to nitrites. Release hydrogen sulphide from proteoses. Do not produce indole from tryptophane. Methyl red reaction negative, and V-P reaction positive. Abundant growth in Koser's citrate medium. Protopectinase not produced. Amylase activity produced by some strains. Proteinase activity very weak or lacking in gelatin and lacking in milk cultures. Fermentation reactions are macroaerogenic in most substrates. In lactose the fermentation reaction is of a slow, acid microaerogenic type. Fresh isolates are pathogenic on *Zea mays*, but pathogenicity is soon lost in pure culture.

The data available concerning *A. dissolvens* may cast a little light on the problem relating to the identity of the "slow lactose-fermenting" coliform bacteria associated with certain intestinal disorders of man discussed by Parr (1939) and Stuart, Griffin, and Baker (1940). It seems that coliform bacteria that are associated with disease of either plants or animals usually exhibit one or more abnormal reactions in culture media,

or seem to lose the ability to effect certain transformations of substrates normally attacked by coliform organisms. Following this line of reasoning, attempts have been made to link the pectolytic and coliform bacteria phytogenetically. The results of this study did not justify such a concept of phylogenetic relationship, because the behavior in culture media and in host plants was so strikingly different in all cases. A number of isolates received as "soft-rot" bacteria were found to exhibit characters typical of coliform bacteria. These were nonpathogenic on the host plants tested and did not produce protopectinase. Six cultures received as *Erwinia carotovora* (W48, W51, W52, W53, CA1, and CA2) and *E. solanisapra* (EP) were readily identified as strains of *Aerobacter aerogenes*. A culture received as *Erwinia phytophthora* (P4) was found to be a strain of *Escherichia freundii*. Culture No. 496, received as *Erwinia phytophthora*, was neither a coliform nor a pectolytic organism. These coliform organisms, apparently isolated from decaying plant material, usually exhibited a slow lactose fermentation.

SUMMARY

Crossinoculation, morphological, cultural, and biochemical studies were made with 78 cultures of peritrichous and nonmotile plant pathogenic bacteria together with a few nonphytopathogenic bacteria to determine their relationships. Eleven peritrichous and one nonmotile plant pathogenic species were studied together with *Aerobacter aerogenes*, *A. cloacae*, *Escherichia coli*, *E. freundii*, *Serratia marcescens*, and *Proteus vulgaris*.

The crossinoculation studies indicated that the cultures of *Erwinia amylovora*, *E. tracheiphila*, and *E. salicis* were highly host specific as compared with the pectolytic bacteria. Two cultures of *E. lathyri* were found to be nonpathogenic on any of the plants inoculated. *Bacterium dissolvens* showed only slight pathogenicity on corn and none on the other plants. A number of nonpathogenic cultures received as species of soft-rot bacteria were identified as species of coliform bacteria.

In morphology and staining reactions the plant-pathogenic bacteria were practically indistinguishable from the nonpathogenic species.

In culture media *Erwinia amylovora*, *E. tracheiphila*, and *E. salicis* produced notably less luxuriant growth than did the other organisms. They either failed to grow or produced small, pale-pink colonies on E.M.B. and Endo-agar slants. *E. salicis* was distinguished by its yellow growth on potato dextrose-agar slants. *E. tracheiphila* produced a slimy growth on all media containing sugar. *E. amylovora* was exceptionally short lived on potato dextrose-agar. The pectolytic organisms grew faster than *E. amylovora* but slower than the coliform bacteria in all culture media. The E.M.B. and Endo-agar colonies of the pectolytic bacteria were relatively small and produced cultural characters indicating their relationship to the species Aerobacter.

The biochemical activities of *Erwinia tracheiphila*, *E. amylovora*, and *E. salicis* in culture media were quite restricted. These species did not

reduce nitrates or produce hydrogen sulphide from proteose peptone broth. *E. amylovora* produced an alkaline reaction in litmus milk with weak proteolysis, whereas *E. tracheiphila* and *E. salicis* produced no visible changes. These bacteria produced no protopectinase or amylase activity. *E. amylovora* produced a rather slow liquefaction of gelatin, the other two species none. Marked acid production for all these organisms was observed only with glucose, fructose, sucrose, and pectin. Certain other carbon compounds were fermented by one species but not by the others, making possible convenient species separations.

The pectolytic bacteria resembled the coliform organisms in many of their biochemical reactions. They differed in that the pectolytic bacteria produced marked protopectinase and gelatinase activity and fermented carbon sources anaerogenically or microaerogenically. The V-P reaction served to distinguish *E. aroideae* from *E. melonis.*

All except one culture of the pectolytic bacteria failed to ferment maltose. Glycerol served to distinguish *E. carotovora* and *E. phytophthora.*

The corn stalk-rot bacteria resembled the coliform bacteria in all respects except their lactose fermentation which was characteristically slow and weak.

The cultures received as *E. lathyri* showed a close relationship to *Serratia marcescens* in their biochemical activities.

Erwinia ananas resembled *E. amylovora* in its nitrate reduction but resembled *Serratia marcescens* somewhat in other respects.

The peritrichous and nonmotile plant pathogenic bacteria were found to consist of four groups of generic rank.

A new family, *Erwiniaceae,* was proposed to include the emended genus *Erwinia* and related genera. The genus *Erwinia* is now conceived as a unit consisting of:

E. amylovora (Burrill) Winslow *et al.,* 1917
E. tracheiphila (E.F.S.) Holland, 1920
E. salicis (Day) Bergey *et al.,* 1939

The genus *Pectobacterium* was proposed for the pectolytic bacteria, and it is proposed to include in it the family *Enterobacteriaceae* with the coliform bacteria. The genus is composed of:

Pectobacterium carotovorum (Jones) Waldee, as type
P. phytophthorum (Frank) Waldee
P. aroideae (Townsend) Waldee
P. melonis (Giddings) Waldee

and a newly described species:

P. delphinii Waldee

The corn stalk-rot bacterium was transferred to the genus *Aerobacter* as:

Aerobacter dissolvens (Rosen) Waldee

The yellow peritrichous plant pathogen bacteria, *Bacterium lathyri* Manns and Taub., *B. ananas* Serrano, occupy a doubtful position taxonomically but seem to be close to *Serratia marcescens.*

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